(54) Title: REGULATION OF HUMAN RTA-LIKE G PROTEIN-COUPLED RECEPTOR

(57) Abstract: Reagents which regulate human RTA-like G protein-coupled receptor can be used to infections such as bacterial, fun-
gal, protozoan, and viral infections, particularly those caused by HIV viruses, pain, cancers, anorexia, bulimia, asthma, Parkinson's
diseases, acute heart failure, hypotension, hypertension, urinary retention, osteoporosis, angina pectoris, myocardial infarction, ul-
cers, asthma, allergies, multiple sclerosis, benign prostatic hypertrophy, and psychotic and neurological disorders, including anxiety,
schizophrenia, manic depression, delirium, dementia, several mental retardation, and dyskinesias, such as Huntington's disease and
Tourett's syndrome.

REGULATION OF HUMAN RTA-LIKE G PROTEIN-COUPLED RECEPTOR

5   ## TECHNICAL FIELD OF THE INVENTION

The invention relates to the area of G protein-coupled receptors. More particularly, it relates to the area of human RTA-like G protein-coupled receptors and their regulation for therapeutic purposes.

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BACKGROUND OF THE INVENTION

G-Protein Coupled Receptors

15   Many medically significant biological processes are mediated by signal transduction pathways that involve G-proteins (Lefkowitz, *Nature 351*, 353-354, 1991). The family of G-protein coupled receptors (GPCR) includes receptors for hormones, neurotransmitters, growth factors, and viruses. Specific examples of GPCRs include receptors for such diverse agents as dopamine, calcitonin, adrenergic hormones,

20   endothelin, cAMP, adenosine, acetylcholine, serotonin, histamine, thrombin, kinin, follicle stimulating hormone, opsins, endothelial differentiation gene-1, rhodopsins, odorants, cytomegalovirus, G-proteins themselves, effector proteins such as phospholipase C, adenyl cyclase, and phosphodiesterase, and actuator proteins such as protein kinase A and protein kinase C.

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GPCRs possess seven conserved membrane-spanning domains connecting at least eight divergent hydrophilic loops. GPCRs (also known as 7TM receptors) have been characterized as including these seven conserved hydrophobic stretches of about 20 to 30 amino acids, connecting at least eight divergent hydrophilic loops. Most

30   GPCRs have single conserved cysteine residues in each of the first two extracellular loops, which form disulfide bonds that are believed to stabilize functional protein

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structure. The seven transmembrane regions are designated as TM1, TM2, TM3, TM4, TM5, TM6, and TM7. TM3 has been implicated in signal transduction.

Phosphorylation and lipidation (palmitylation or farnesylation) of cysteine residues
5   can influence signal transduction of some GPCRs. Most GPCRs contain potential phosphorylation sites within the third cytoplasmic loop and/or the carboxy terminus. For several GPCRs, such as the μ-adrenergic receptor, phosphorylation by protein kinase A and/or specific receptor kinases mediates receptor desensitization.

10   For some receptors, the ligand binding sites of GPCRs are believed to comprise hydrophilic sockets formed by several GPCR transmembrane domains. The hydro-philic sockets are surrounded by hydrophobic residues of the GPCRs. The hydrophilic side of each GPCR transmembrane helix is postulated to face inward and form a polar ligand binding site. TM3 has been implicated in several GPCRs as
15   having a ligand binding site, such as the TM3 aspartate residue. TM5 serines, a TM6 asparagine, and TM6 or TM7 phenylalanines or tyrosines also are implicated in ligand binding.

GPCRs are coupled inside the cell by heterotrimeric G-proteins to various
20   intracellular enzymes, ion channels, and transporters (*see* Johnson *et al.*, *Endoc. Rev. 10*, 317-331, 1989). Different G-protein alpha-subunits preferentially stimulate particular effectors to modulate various biological functions in a cell. Phosphorylation of cytoplasmic residues of GPCRs is an important mechanism for the regulation of some GPCRs. For example, in one form of signal transduction, the
25   effect of hormone binding is the activation inside the cell of the enzyme, adenylate cyclase. Enzyme activation by hormones is dependent on the presence of the nucleotide GTP. GTP also influences hormone binding. A G-protein connects the hormone receptor to adenylate cyclase. G-protein exchanges GTP for bound GDP when activated by a hormone receptor. The GTP-carrying form then binds to
30   activated adenylate cyclase. Hydrolysis of GTP to GDP, catalyzed by the G-protein itself, returns the G-protein to its basal, inactive form. Thus, the G-protein serves a

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dual role, as an intermediate that relays the signal from receptor to effector, and as a clock that controls the duration of the signal.

5   Over the past 15 years, nearly 350 therapeutic agents targeting GPCRs receptors have been successfully introduced onto the market. This indicates that these receptors have an established, proven history as therapeutic targets. Clearly, there is an on-going need for identification and characterization of further GPCRs which can play a role in preventing, ameliorating, or correcting dysfunctions or diseases including, but not limited to, infections such as bacterial, fungal, protozoan, and viral infections,

10   particularly those caused by HIV viruses, pain, cancers, anorexia, bulimia, asthma, Parkinson's diseases, acute heart failure, hypotension, hypertension, urinary retention, osteoporosis, angina pectoris, myocardial infarction, ulcers, asthma, allergies, benign prostatic hypertrophy, and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, several

15   mental retardation, and dyskinesias, such as Huntington's disease and Tourett's syndrome.

Because of the wide-spread distribution of GCPRs with diverse biological effects, there is a need in the art to identify additional members of the GCPR family whose

20   activity can be regulated to provide therapeutic effects.

SUMMARY OF THE INVENTION

It is an object of the invention to provide reagents and methods of regulating a

25   human RTA-like G protein-coupled receptor (RTA-like-GPCR). This and other objects of the invention are provided by one or more of the embodiments described below.

One embodiment of the invention is a RTA-like-GPCR polypeptide comprising an

30   amino acid sequence selected from the group consisting of:

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amino acid sequences which are at least about 50% identical to the amino acid sequence shown in SEQ ID NO: 2; and

the amino acid sequence shown in SEQ ID NO: 2.

Yet another embodiment of the invention is a method of screening for agents which decrease the activity of RTA-like-GPCR. A test compound is contacted with a RTA-like-GPCR polypeptide comprising an amino acid sequence selected from the group consisting of:

amino acid sequences which are at least about 50% identical to the amino acid sequence shown in SEQ ID NO: 2; and

the amino acid sequence shown in SEQ ID NO: 2.

Binding between the test compound and the RTA-like-GPCR polypeptide is detected. A test compound which binds to the RTA-like-GPCR polypeptide is thereby identified as a potential agent for decreasing the activity of RTA-like-GPCR.

Another embodiment of the invention is a method of screening for agents which decrease the activity of RTA-like-GPCR. A test compound is contacted with a poly-nucleotide encoding a RTA-like-GPCR polypeptide, wherein the polynucleotide comprises a nucleotide sequence selected from the group consisting of:

nucleotide sequences which are at least about 50% identical to the nucleotide sequence shown in SEQ ID NO: 1; and

the nucleotide sequence shown in SEQ ID NO: 1.

Binding of the test compound to the polynucleotide is detected. A test compound which binds to the polynucleotide is identified as a potential agent for decreasing the

activity of RTA-like-GPCR. The agent can work by decreasing the amount of the RTA-like-GPCR through interacting with the RTA-like-GPCR mRNA.

Another embodiment of the invention is a method of screening for agents which
5   regulate the activity of RTA-like-GPCR. A test compound is contacted with a RTA-like-GPCR polypeptide comprising an amino acid sequence selected from the group consisting of:

        amino acid sequences which are at least about 50% identical to the amino acid
10   sequence shown in SEQ ID NO: 2; and

        the amino acid sequence shown in SEQ ID NO: 2.

        A RTA-like-GPCR activity of the polypeptide is detected. A test compound which
15   increases RTA-like-GPCR activity of the polypeptide relative to RTA-like-GPCR activity in the absence of the test compound is thereby identified as a potential agent for increasing the activity of RTA-like-GPCR. A test compound which decreases RTA-like-GPCR activity of the polypeptide relative to RTA-like-GPCR activity in the absence of the test compound is thereby identified as a potential agent for
20   decreasing the activity of RTA-like-GPCR.

        Even another embodiment of the invention is a method of screening for agents which decrease the activity of RTA-like-GPCR. A test compound is contacted with a RTA-like-GPCR product of a polynucleotide which comprises a nucleotide sequence
25   selected from the group consisting of:

        nucleotide sequences which are at least about 50% identical to the nucleotide sequence shown in SEQ ID NO: 1; and

30   the nucleotide sequence shown in SEQ ID NO: 1.

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Binding of the test compound to the RTA-like-GPCR product is detected. A test compound which binds to the RTA-like-GPCR product is thereby identified as a potential agent for decreasing the activity of RTA-like-GPCR.

5   Still another embodiment of the invention is a method of reducing the activity of RTA-like-GPCR. A cell is contacted with a reagent which specifically binds to a polynucleotide encoding a RTA-like-GPCR polypeptide or the product encoded by the polynucleotide, wherein the polynucleotide comprises a nucleotide sequence selected from the group consisting of:

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nucleotide sequences which are at least about 50% identical to the nucleotide sequence shown in SEQ ID NO: 1; and

the nucleotide sequence shown in SEQ ID NO: 1.

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RTA-like-GPCR activity in the cell is thereby decreased.

The invention thus provides an RTA-like G protein-coupled receptor which can be used to identify RTA analogs as well as compounds which may act as somatostatin

20   antagonists at the receptor site. RTA-like G-protein coupled receptor and fragments thereof also are useful in raising specific antibodies which can block the receptor.

BRIEF DESCRIPTION OF THE DRAWING

25   Fig. 1 shows the DNA-sequence encoding a RTA-like-GPCR polypeptide.
Fig. 2 shows the amino acid sequence deduced from the DNA-sequence of Fig.1.

DETAILED DESCRIPTION OF THE INVENTION

The invention relates to an isolated polynucleotide encoding a RTA-like-GPCR polypeptide and being selected from the group consisting of:

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a)   a polynucleotide encoding a RTA-like-GPCR polypeptide comprising an amino acid sequence selected from the group consisting of:

amino acid sequences which are at least about 50% identical to

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the amino acid sequence shown in SEQ ID NO: 2; and

the amino acid sequence shown in SEQ ID NO: 2;

15   b)   a polynucleotide comprising the sequence of SEQ ID NO: 1;

c)   a polynucleotide which hybridizes under stringent conditions to a poly-nucleotide specified in (a) and (b);

20   d)   a polynucleotide the sequence of which deviates from the polynucleotide sequences specified in (a) to (c) due to the degeneration of the genetic code; and

e)   a polynucleotide which represents a fragment, derivative or allelic variation of

25   a polynucleotide sequence specified in (a) to (d).

Furthermore, it has been discovered by the present applicant that a RTA-like G protein-coupled receptor (RTA-like-GPCR), particularly a human RTA-like-GPCR, and agents which regulate it can be used in therapeutic methods to treat disorders

30   such as bacterial, fungal, protozoan, and viral infections, particularly those caused by HIV viruses, pain, cancers, anorexia, bulimia, asthma, Parkinson's diseases, acute

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heart failure, hypotension, hypertension, urinary retention, osteoporosis, angina pectoris, myocardial infarction, ulcers, asthma, allergies, benign prostatic hypertrophy, and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, several mental retardation, and dyskinesias,

5   such as Huntington's disease and Tourett's syndrome. Human RTA-like GPCR also can be used to screen for receptor agonists and antagonists.

RTA-like-GPCR polypeptides according to the invention comprise an amino acid sequence shown in SEQ ID NO:2, a portion of that sequence, or a biologically active

10   variant thereof, as defined below. An RTA-like-GPCR polypeptide of the invention therefore can be a portion of an RTA-like-GPCR protein, a full-length RTA-like-GPCR protein, or a fusion protein comprising all or a portion of an RTA-like-GPCR protein. An amino acid sequence of human RTA-like GPCR is shown in SEQ ID NO:2. Transmembrane helices are present from amino acids 32 to 54, 62 to 82, 116

15   to 138, 152 to 173, 192 to 214, 227 to 249, and 262 to 288.

Biologically Active Variants

RTA-like-GPCR polypeptide variants which are biologically active, _i.e._, retain the

20   ability to bind a ligand to produce a biological effect, such as cyclic AMP formation, mobilization of intracellular calcium, or phosphoinositide metabolism, also are RTA-like- GPCR polypeptides. Preferably, naturally or non-naturally occurring RTA-like-GPCR polypeptide variants have amino acid sequences which are at least about 50, preferably about 75, 90, 96, or 98% identical to an amino acid sequence shown in

25   SEQ ID NO:2 or a fragment thereof. Percent identity between a putative RTA-like-GPCR polypeptide variant and an amino acid sequence of SEQ ID NO:2 is determined using the Blast2 alignment program.

Variations in percent identity can be due, for example, to amino acid substitutions,

30   insertions, or deletions. Amino acid substitutions are defined as one for one amino acid replacements. They are conservative in nature when the substituted amino acid

has similar structural and/or chemical properties. Examples of conservative replacements are substitution of a leucine with an isoleucine or valine, an aspartate with a glutamate, or a threonine with a serine.

5   Amino acid insertions or deletions are changes to or within an amino acid sequence. They typically fall in the range of about 1 to 5 amino acids. Guidance in determining which amino acid residues can be substituted, inserted, or deleted without abolishing biological or immunological activity of an RTA-like-GPCR polypeptide can be found using computer programs well known in the art, such as DNASTAR software.

10   Whether an amino acid change results in a biologically active RTA-like-GPCR polypeptide can readily be determined by assaying for binding to a ligand or by conducting a functional assay, as described for example, in the specific Examples, below.

15   *Fusion Proteins*

Fusion proteins can comprise at least 5, 6, 8, 10, 25, or 50 or RTA-like contiguous amino acids of an amino acid sequence shown in SEQ ID NO:2. Fusion proteins are useful for generating antibodies against RTA-like-GPCR polypeptide amino acid

20   sequences and for use in various assay systems. For example, fusion proteins can be used to identify proteins which interact with portions of an RTA-like-GPCR polypeptide. Protein affinity chromatography or library-based assays for protein-protein interactions, such as the yeast two-hybrid or phage display systems, can be used for this purpose. Such methods are well known in the art and also can be used

25   as drug screens.

An RTA-like-GPCR polypeptide fusion protein comprises two polypeptide segments fused together by means of a peptide bond. The first polypeptide segment comprises at least 5, 6, 8, 10, 25, or 50 or RTA-like contiguous amino acids of SEQ ID NO:2

30   or from a biologically active variant, such as those described above. The first polypeptide segment also can comprise full-length RTA-like-GPCR protein.

The second polypeptide segment can be a full-length protein or a protein fragment. Proteins commonly used in fusion protein construction include β-galactosidase, β-glucuronidase, green fluorescent protein (GFP), autofluorescent proteins, including blue fluorescent protein (BFP), glutathione-S-transferase (GST), luciferase, horseradish peroxidase (HRP), and chloramphenicol acetyltransferase (CAT). Additionally, epitope tags are used in fusion protein constructions, including histidine (His) tags, FLAG tags, influenza hemagglutinin (HA) tags, Myc tags, VSV-G tags, and thioredoxin (Trx) tags. Other fusion constructions can include maltose binding protein (MBP), S-tag, Lex a DNA binding domain (DBD) fusions, GAL4 DNA binding domain fusions, and herpes simplex virus (HSV) BP16 protein fusions. A fusion protein also can be engineered to contain a cleavage site located between the RTA-like-GPCR polypeptide-encoding sequence and the heterologous protein sequence, so that the RTA-like-GPCR polypeptide can be cleaved and purified away from the heterologous moiety.

A fusion protein can be synthesized chemically, as is known in the art. Preferably, a fusion protein is produced by covalently linking two polypeptide segments or by standard procedures in the art of molecular biology. Recombinant DNA methods can be used to prepare fusion proteins, for example, by making a DNA construct which comprises coding sequences selected from SEQ ID NO:1 in proper reading frame with nucleotides encoding the second polypeptide segment and expressing the DNA construct in a host cell, as is known in the art. Many kits for constructing fusion proteins are available from companies such as Promega Corporation (Madison, WI), Stratagene (La Jolla, CA), CLONTECH (Mountain View, CA), Santa Cruz Biotechnology (Santa Cruz, CA), MBL International Corporation (MIC; Watertown, MA), and Quantum Biotechnologies (Montreal, Canada; 1-888-DNA-KITS).

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Identification of Species Homologs

Species homologs of human RTA-like-GPCR polypeptide can be obtained using RTA-like-GPCR polypeptide polynucleotides (described below) to make suitable probes or primers for screening cDNA expression libraries from other species, such as mice, monkeys, or yeast, identifying cDNAs which encode homologs of RTA-like-GPCR polypeptide, and expressing the cDNAs as is known in the art.

RTA-like-GPCR Polynucleotides

An RTA-like-GPCR polynucleotide can be single- or double-stranded and comprises a coding sequence or the complement of a coding sequence for an RTA-like-GPCR polypeptide. A coding sequence for human RTA-like-GPCR is shown in SEQ ID NO:1.

Degenerate nucleotide sequences encoding human RTA-like-GPCR polypeptides, as well as homologous nucleotide sequences which are at least about 50, preferably about 75, 90, 96, or 98% identical to the nucleotide sequence shown in SEQ ID NO:1 also are RTA-like-GPCR polynucleotides. Percent sequence identity between the sequences of two polynucleotides is determined using computer programs such as ALIGN which employ the FASTA algorithm, using an affine gap search with a gap open penalty of -12 and a gap extension penalty of -2. Complementary DNA (cDNA) molecules, species homologs, and variants of RTA-like-GPCR poly-nucleotides which encode biologically active RTA-like-GPCR polypeptides also are RTA-like-GPCR polynucleotides.

Identification of Variants and Homologs of RTA-like-GPCR Polynucleotides

Variants and homologs of the RTA-like-GPCR polynucleotides described above also are RTA-like-GPCR polynucleotides. Typically, homologous RTA-like-GPCR poly-nucleotide sequences can be identified by hybridization of candidate polynucleotides

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to known RTA-like-GPCR polynucleotides under stringent conditions, as is known in the art. For example, using the following wash conditions--2X SSC (0.3 M NaCl, 0.03 M sodium citrate, pH 7.0), 0.1% SDS, room temperature twice, 30 minutes each; then 2X SSC, 0.1% SDS, 50 °C once, 30 minutes; then 2X SSC, room temperature twice, 10 minutes each--homologous sequences can be identified which contain at most about 25-30% basepair mismatches. RTA-like preferably, homologous nucleic acid strands contain 15-25% basepair mismatches, even more preferably 5-15% basepair mismatches.

Species homologs of the RTA-like-GPCR polynucleotides disclosed herein also can be identified by making suitable probes or primers and screening cDNA expression libraries from other species, such as mice, monkeys, or yeast. Human variants of RTA-like-GPCR polynucleotides can be identified, for example, by screening human cDNA expression libraries. It is well known that the T_m of a double-stranded DNA decreases by 1-1.5 °C with every 1% decrease in homology (Bonner *et al.*, *J. Mol. Biol. 81*, 123 (1973). Variants of human RTA-like-GPCR polynucleotides or RTA-like-GPCR polynucleotides of other species can therefore be identified by hybridizing a putative homologous RTA-like-GPCR polynucleotide with a polynucleotide having a nucleotide sequence of SEQ ID NO:1   or the complement thereof to form a test hybrid. The melting temperature of the test hybrid is compared with the melting temperature of a hybrid comprising transformylase polynucleotides having perfectly complementary nucleotide sequences, and the number or percent of basepair mismatches within the test hybrid is calculated.

Nucleotide sequences which hybridize to transformylase polynucleotides or their complements following stringent hybridization and/or wash conditions also are RTA-like-GPCR polynucleotides. Stringent wash conditions are well known and understood in the art and are disclosed, for example, in Sambrook *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL, 2d ed., 1989, at pages 9.50-9.51.

Typically, for stringent hybridization conditions a combination of temperature and salt concentration should be chosen that is approximately 12-20 °C below the calculated T_m of the hybrid under study. The T_m of a hybrid between an RTA-like-GPCR polynucleotide having a nucleotide sequence shown in SEQ ID NO:1 or the complement thereof and a polynucleotide sequence which is at least about 50, preferably about 75, 90, 96, or 98% identical to one of those nucleotide sequences can be calculated, for example, using the equation of Bolton and McCarthy, *Proc. Natl. Acad. Sci. U.S.A. 48*, 1390 (1962):

$$T_m = 81.5\ °C - 16.6(\log_{10}[Na^+]) + 0.41(\%G + C) - 0.63(\%formamide) - 600/l),$$

where l = the length of the hybrid in basepairs.

Stringent wash conditions include, for example, 4X SSC at 65 °C, or 50% formamide, 4X SSC at 42 °C, or 0.5X SSC, 0.1% SDS at 65 °C. Highly stringent wash conditions include, for example, 0.2X SSC at 65 °C.

Preparation of RTA-like-GPCR Polynucleotides

A naturally occurring RTA-like-GPCR polynucleotide can be isolated free of other cellular components such as membrane components, proteins, and lipids. Polynucleotides can be made by a cell and isolated using standard nucleic acid purification techniques, or synthesized using an amplification technique, such as the polymerase chain reaction (PCR), or by using an automatic synthesizer. Methods for isolating polynucleotides are routine and are known in the art. Any such technique for obtaining a polynucleotide can be used to obtain isolated RTA-like-GPCR polynucleotides. For example, restriction enzymes and probes can be used to isolate polynucleotide fragments which comprises RTA-like-GPCR nucleotide sequences. Isolated polynucleotides are in preparations which are free or at least 70, 80, or 90% free of other molecules.

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RTA-like oncogene-related-GPCR cDNA molecules can be made with standard molecular biology techniques, using RTA-like-GPCR mRNA as a template. RTA-like-GPCR cDNA molecules can thereafter be replicated using molecular biology techniques known in the art and disclosed in manuals such as Sambrook *et al.* (1989).

5   An amplification technique, such as PCR, can be used to obtain additional copies of polynucleotides of the invention, using either human genomic DNA or cDNA as a template.

Alternatively, synthetic chemistry techniques can be used to synthesizes RTA-like-

10   GPCR polynucleotides. The degeneracy of the genetic code allows alternate nucleotide sequences to be synthesized which will encode an RTA-like-GPCR polypeptide having, for example, an amino acid sequence shown in SEQ ID NO:2 or a biologically active variant thereof.

15   *Extending RTA-like-GPCR Polynucleotides*

Various PCR-based methods can be used to extend the nucleic acid sequences encoding the disclosed portions of human RTA-like-GPCR polypeptide to detect upstream sequences such as promoters and regulatory elements. For example,

20   restriction-site PCR uses universal primers to retrieve unknown sequence adjacent to a known locus (Sarkar, *PCR Methods Applic. 2*, 318-322, 1993). Genomic DNA is first amplified in the presence of a primer to a linker sequence and a primer specific to the known region. The amplified sequences are then subjected to a second round of PCR with the same linker primer and another specific primer internal to the first

25   one. Products of each round of PCR are transcribed with an appropriate RNA polymerase and sequenced using reverse transcriptase.

Inverse PCR also can be used to amplify or extend sequences using divergent primers based on a known region (Triglia *et al.*, *Nucleic Acids Res. 16*, 8186, 1988). Primers

30   can be designed using commercially available software, such as OLIGO 4.06 Primer Analysis software (National Biosciences Inc., Plymouth, Minn.), to be 22-30

nucleotides in length, to have a GC content of 50% or more, and to anneal to the target sequence at temperatures about 68-72 °C. The method uses several restriction enzymes to generate a suitable fragment in the known region of a gene. The fragment is then circularized by intramolecular ligation and used as a PCR template.

5

Another method which can be used is capture PCR, which involves PCR amplification of DNA fragments adjacent to a known sequence in human and yeast artificial chromosome DNA (Lagerstrom *et al.*, *PCR Methods Applic. 1*, 111-119, 1991). In this method, multiple restriction enzyme digestions and ligations also can

10   be used to place an engineered double-stranded sequence into an unknown fragment of the DNA molecule before performing PCR.

Another method which can be used to retrieve unknown sequences is that of Parker *et al.*, *Nucleic Acids Res. 19*, 3055-3060, 1991). Additionally, PCR, nested primers,

15   and PROMOTERFINDER libraries (CLONTECH, Palo Alto, Calif.) can be used to walk genomic DNA (CLONTECH, Palo Alto, Calif.). This process avoids the need to screen libraries and is useful in finding intron/exon junctions.

When screening for full-length cDNAs, it is preferable to use libraries that have been

20   size-selected to include larger cDNAS. Randomly-primed libraries are preferable, in that they will contain more sequences which contain the 5' regions of genes. Use of a randomly primed library may be especially preferable for situations in which an oligo d(T) library does not yield a full-length cDNA. Genomic libraries can be useful for extension of sequence into 5' non-transcribed regulatory regions.

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Commercially available capillary electrophoresis systems can be used to analyze the size or confirm the nucleotide sequence of PCR or sequencing products. For example, capillary sequencing can employ flowable polymers for electrophoretic separation, four different fluorescent dyes (one for each nucleotide) which are laser

30   activated, and detection of the emitted wavelengths by a charge coupled device camera. Output/light intensity can be converted to electrical signal using appropriate

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software (e.g. GENOTYPER and Sequence NAVIGATOR, Perkin Elmer), and the entire process from loading of samples to computer analysis and electronic data display can be computer controlled. Capillary electrophoresis is especially preferable for the sequencing of small pieces of DNA which might be present in limited amounts in a particular sample.

Obtaining RTA-like-GPCR Polypeptides

RTA-like-GPCR polypeptides can be obtained, for example, by purification from human cells, by expression of RTA-like-GPCR polynucleotides, or by direct chemical synthesis.

Protein Purification

RTA-like-GPCR polypeptides can be purified from any human cell which expresses the receptor, including host cells which have been transfected with RTA-like-GPCR polynucleotides. Gut, vas deferens, uterus, aorta, and cerebellum are particularly useful sources of RTA-like-GPCR polypeptides. A purified RTA-like-GPCR poly-peptide is separated from other compounds which normally associate with the RTA-like-GPCR polypeptide in the cell, such as certain proteins, carbohydrates, or lipids, using methods well-known in the art. Such methods include, but are not limited to, size exclusion chromatography, ammonium sulfate fractionation, ion exchange chromatography, affinity chromatography, and preparative gel electrophoresis.

RTA-like-GPCR polypeptide can be conveniently isolated as a complex with its associated G protein, as described in the specific examples, below. A preparation of purified RTA-like-GPCR polypeptides is at least 80% pure; preferably, the preparations are 90%, 95%, or 99% pure. Purity of the preparations can be assessed by any means known in the art, such as SDS-polyacrylamide gel electrophoresis.

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Expression of RTA-like-GPCR Polynucleotides

To express an RTA-like-GPCR polypeptide, an RTA-like-GPCR polynucleotide can be inserted into an expression vector which contains the necessary elements for the transcription and translation of the inserted coding sequence. Methods which are well known to those skilled in the art can be used to construct expression vectors containing sequences encoding RTA-like-GPCR polypeptides and appropriate transcriptional and translational control elements. These methods include *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. Such techniques are described, for example, in Sambrook *et al.* (1989) and in Ausubel *et al.*, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, New York, N.Y., 1989.

A variety of expression vector/host systems can be utilized to contain and express sequences encoding an RTA-like-GPCR polypeptide. These include, but are not limited to, microorganisms, such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with yeast expression vectors, insect cell systems infected with virus expression vectors (*e.g.*, baculovirus), plant cell systems transformed with virus expression vectors (*e.g.*, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or with bacterial expression vectors (*e.g.*, Ti or pBR322 plasmids), or animal cell systems.

The control elements or regulatory sequences are those non-translated regions of the vector -- enhancers, promoters, 5' and 3' untranslated regions -- which interact with host cellular proteins to carry out transcription and translation. Such elements can vary in their strength and specificity. Depending on the vector system and host utilized, any number of suitable transcription and translation elements, including constitutive and inducible promoters, can be used. For example, when cloning in bacterial systems, inducible promoters such as the hybrid lacZ promoter of the BLUESCRIPT phagemid (Stratagene, LaJolla, Calif.) or pSPORT1 plasmid (Life Technologies) and the like can be used. The baculovirus polyhedrin promoter can

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be used in insect cells. Promoters or enhancers derived from the genomes of plant cells (*e.g.*, heat shock, RUBISCO, and storage protein genes) or from plant viruses (*e.g.*, viral promoters or leader sequences) can be cloned into the vector. In mammalian cell systems, promoters from mammalian genes or from mammalian viruses are preferable. If it is necessary to generate a cell line that contains multiple copies of a nucleotide sequence encoding an RTA-like-GPCR polypeptide, vectors based on SV40 or EBV can be used with an appropriate selectable marker.

Bacterial and Yeast Expression Systems

In bacterial systems, a number of expression vectors can be selected depending upon the use intended for the RTA-like-GPCR polypeptide. For example, when a large quantity of an RTA-like-GPCR polypeptide is needed for the induction of anti-bodies, vectors which direct high level expression of fusion proteins that are readily purified can be used. Such vectors include, but are not limited to, multifunctional *E. coli* cloning and expression vectors such as BLUESCRIPT (Stratagene). In a BLUESCRIPT vector, a sequence encoding the RTA-like-GPCR polypeptide can be ligated into the vector in frame with sequences for the amino-terminal Met and the subsequent 7 residues of β-galactosidase so that a hybrid protein is produced. pIN vectors (Van Heeke & Schuster, *J. Biol. Chem.* 264, 5503-5509, 1989) or pGEX vectors (Promega, Madison, Wis.) also can be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. Proteins made in such systems can be designed to include heparin, thrombin, or factor Xa protease cleavage sites so that the cloned polypeptide of interest can be released from the GST moiety at will.

In the yeast *Saccharomyces cerevisiae*, a number of vectors containing constitutive or inducible promoters such as alpha factor, alcohol oxidase, and PGH can be used.

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For reviews, see Ausubel *et al.* (1989) and Grant *et al.*, *Methods Enzymol. 153*, 516-544, 1987.

Plant and Insect Expression Systems

5

If plant expression vectors are used, the expression of sequences encoding RTA-like-GPCR polypeptides can be driven by any of a number of promoters. For example, viral promoters such as the 35S and 19S promoters of CaMV can be used alone or in combination with the omega leader sequence from TMV (Takamatsu, *EMBO J. 6*,

10   307-311, 1987). Alternatively, plant promoters such as the small subunit of RUBISCO or heat shock promoters can be used (Coruzzi *et al.*, *EMBO J. 3*, 1671-1680, 1984; Broglie *et al.*, *Science 224*, 838-843, 1984; Winter *et al.*, *Results Probl. Cell Differ. 17*, 85-105, 1991). These constructs can be introduced into plant cells by direct DNA transformation or by pathogen-mediated transfection. Such techniques

15   are described in a number of generally available reviews (*e.g.*, Hobbs or Murray, in MCGRAW HILL YEARBOOK OF SCIENCE AND TECHNOLOGY, McGraw Hill, New York, N.Y., pp. 191-196, 1992).

An insect system also can be used to express an RTA-like-GPCR polypeptide. For

20   example, in one such system *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes in *Spodoptera frugiperda* cells or in *Trichoplusia* larvae. Sequences encoding RTA-like-GPCR polypeptides can be cloned into a non-essential region of the virus, such as the polyhedrin gene, and placed under control of the polyhedrin promoter. Successful insertion of RTA-like-

25   GPCR polypeptides will render the polyhedrin gene inactive and produce recombinant virus lacking coat protein. The recombinant viruses can then be used to infect *S. frugiperda cells* or *Trichoplusia* larvae in which RTA-like-GPCR polypeptides can be expressed (Engelhard *et al.*, *Proc. Nat. Acad. Sci. 91*, 3224-3227, 1994).

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Mammalian Expression Systems

A number of viral-based expression systems can be used to express RTA-like-GPCR polypeptides in mammalian host cells. For example, if an adenovirus is used as an

5   expression vector, sequences encoding RTA-like-GPCR polypeptides can be ligated into an adenovirus transcription/translation complex comprising the late promoter and tripartite leader sequence. Insertion in a non-essential E1 or E3 region of the viral genome can be used to obtain a viable virus which is capable of expressing an RTA-like-GPCR polypeptide in infected host cells (Logan & Shenk, *Proc. Natl.*

10   *Acad. Sci. 81*, 3655-3659, 1984). If desired, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, can be used to increase expression in mammalian host cells.

Human artificial chromosomes (HACs) also can be used to deliver larger fragments

15   of DNA than can be contained and expressed in a plasmid. HACs of 6M to 10M are constructed and delivered to cells via conventional delivery methods (*e.g.*, liposomes, polycationic amino polymers, or vesicles).

Specific initiation signals also can be used to achieve more efficient translation of

20   sequences encoding RTA-like-GPCR polypeptides. Such signals include the ATG initiation codon and adjacent sequences. In cases where sequences encoding an RTA-like-GPCR polypeptide, its initiation codon, and upstream sequences are inserted into the appropriate expression vector, no additional transcriptional or translational control signals may be needed. However, in cases where only coding

25   sequence, or a fragment thereof, is inserted, exogenous translational control signals (including the ATG initiation codon) should be provided. The initiation codon should be in the correct reading frame to ensure translation of the entire insert. Exogenous translational elements and initiation codons can be of various origins, both natural and synthetic. The efficiency of expression can be enhanced by the

30   inclusion of enhancers which are appropriate for the particular cell system which is used (see Scharf *et al.*, *Results Probl. Cell Differ. 20*, 125-162, 1994).

Host Cells

A host cell strain can be chosen for its ability to modulate the expression of the
inserted sequences or to process the expressed RTA-like-GPCR polypeptide in the
desired fashion. Such modifications of the polypeptide include, but are not limited
to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and
acylation. Post-translational processing which cleaves a "prepro" form of the
polypeptide also can be used to facilitate correct insertion, folding and/or function.
Different host cells which have specific cellular machinery and characteristic
mechanisms for post-translational activities (*e.g.*, CHO, HeLa, MDCK, HEK293, and
WI38), are available from the American Type Culture Collection (ATCC; 10801
University Boulevard, Manassas, VA 20110-2209) and can be chosen to ensure the
correct modification and processing of the foreign protein.

Stable expression is preferred for long-term, high-yield production of recombinant
proteins. For example, cell lines which stably express RTA-like-GPCR polypeptides
can be transformed using expression vectors which can contain viral origins of
replication and/or endogenous expression elements and a selectable marker gene on
the same or on a separate vector. Following the introduction of the vector, cells can
be allowed to grow for 1-2 days in an enriched medium before they are switched to a
selective medium. The purpose of the selectable marker is to confer resistance to
selection, and its presence allows growth and recovery of cells which successfully
express the introduced RTA-like-GPCR sequences. Resistant clones of stably trans-
formed cells can be proliferated using tissue culture techniques appropriate to the cell
type. See, for example, ANIMAL CELL CULTURE, R.I. Freshney, ed., 1986.

Any number of selection systems can be used to recover transformed cell lines.

These include, but are not limited to, the herpes simplex virus thymidine kinase
(Wigler *et al.*, *Cell 11*, 223-32, 1977) and adenine ph sphoribosyltransferase (Lowy

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et al., Cell 22, 817-23, 1980) genes which can be employed in *tk⁻* or *aprt⁻* cells, respectively. Also, antimetabolite, antibiotic, or herbicide resistance can be used as the basis for selection. For example, *dhfr* confers resistance to methotrexate (Wigler *et al., Proc. Natl. Acad. Sci. 77*, 3567-70, 1980), *npt* confers resistance to the amino-

5   glycosides, neomycin and G-418 (Colbere-Garapin *et al., J. Mol. Biol. 150*, 1-14, 1981), and *als* and *pat* confer resistance to chlorsulfuron and phosphinotricin acetyl-transferase, respectively (Murray, 1992, *supra*). Additional selectable genes have been described. For example, *trpB* allows cells to utilize indole in place of trypto-phan, or *hisD*, which allows cells to utilize histinol in place of histidine (Hartman &

10   Mulligan, *Proc. Natl. Acad. Sci. 85*, 8047-51, 1988). Visible markers such as anthocyanins, β-glucuronidase and its substrate GUS, and luciferase and its substrate luciferin, can be used to identify transformants and to quantify the amount of transient or stable protein expression attributable to a specific vector system (Rhodes *et al., Methods Mol. Biol. 55*, 121-131, 1995).

15

Detecting Expression of RTA-like-GPCR Polypeptides

Although the presence of marker gene expression suggests that the RTA-like-GPCR polynucleotide is also present, its presence and expression may need to be confirmed.

20   For example, if a sequence encoding an RTA-like-GPCR polypeptide is inserted within a marker gene sequence, transformed cells containing sequences which encode an RTA-like-GPCR polypeptide can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with a sequence encoding an RTA-like-GPCR polypeptide under the control of a single promoter.

25   Expression of the marker gene in response to induction or selection usually indicates expression of the RTA-like-GPCR polynucleotide.

Alternatively, host cells which contain an RTA-like-GPCR polynucleotide and which express an RTA-like-GPCR polypeptide can be identified by a variety of

30   procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridizations and protein bioassay or

immunoassay techniques which include membrane, solution, or chip-based techno-
logies for the detection and/or quantification of nucleic acid or protein. For example,
the presence of a polynucleotide sequence encoding an RTA-like-GPCR polypeptide
can be detected by DNA-DNA or DNA-RNA hybridization or amplification using

5   probes or fragments or fragments of polynucleotides encoding an RTA-like-GPCR
polypeptide. Nucleic acid amplification-based assays involve the use of oligo-
nucleotides selected from sequences encoding an RTA-like-GPCR polypeptide to
detect transformants which contain an RTA-like-GPCR polynucleotide.

10   A variety of protocols for detecting and measuring the expression of an RTA-like-
GPCR polypeptide, using either polyclonal or monoclonal antibodies specific for the
polypeptide, are known in the art. Examples include enzyme-linked immunosorbent
assay (ELISA), radioimmunoassay (RIA), and fluorescence activated cell sorting
(FACS). A two-site, monoclonal-based immunoassay using monoclonal antibodies

15   reactive to two non-interfering epitopes on an RTA-like-GPCR polypeptide can be
used, or a competitive binding assay can be employed. These and other assays are
described in Hampton *et al.*, SEROLOGICAL METHODS: A LABORATORY MANUAL,
APS Press, St. Paul, Minn., 1990) and Maddox *et al.*, *J. Exp. Med. 158*, 1211-1216,
1983).

20
A wide variety of labels and conjugation techniques are known by those skilled in the
art and can be used in various nucleic acid and amino acid assays. Means for
producing labeled hybridization or PCR probes for detecting sequences related to
polynucleotides encoding RTA-like-GPCR polypeptides include oligolabeling, nick

25   translation, end-labeling, or PCR amplification using a labeled nucleotide.
Alternatively, sequences encoding an RTA-like-GPCR polypeptide can be cloned
into a vector for the production of an mRNA probe. Such vectors are known in the
art, are commercially available, and can be used to synthesize RNA probes *in vitro*
by addition of labeled nucleotides and an appropriate RNA polymerase such as T7,

30   T3, or SP6. These procedures can be conducted using a variety of commercially
available kits (Amersham Pharmacia Biotech, Promega, and US Biochemical).

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Suitable reporter molecules or labels which can be used for ease of detection include radionuclides, enzymes, and fluorescent, chemiluminescent, or chromogenic agents, as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

5   *Expression and Purification of RTA-like-GPCR Polypeptides*

Host cells transformed with nucleotide sequences encoding an RTA-like-GPCR polypeptide can be cultured under conditions suitable for the expression and recovery of the protein from cell culture. The polypeptide produced by a transformed cell can
10   be secreted or contained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides which encode RTA-like-GPCR polypeptides can be designed to contain signal sequences which direct secretion of soluble RTA-like-GPCR polypeptides through a prokaryotic or eukaryotic cell membrane or which direct the
15   membrane insertion of membrane-bound RTA-like-GPCR polypeptide.

As discussed above, other constructions can be used to join a sequence encoding an RTA-like-GPCR polypeptide to a nucleotide sequence encoding a polypeptide domain which will facilitate purification of soluble proteins. Such purification
20   facilitating domains include, but are not limited to, metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilized metals, protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp., Seattle, Wash.). Inclusion of cleavable linker sequences such as those specific for
25   Factor Xa or enterokinase (Invitrogen, San Diego, CA) between the purification domain and the RTA-like-GPCR polypeptide also can be used to facilitate purification. One such expression vector provides for expression of a fusion protein containing an RTA-like-GPCR polypeptide and 6 histidine residues preceding a thioredoxin or an enterokinase cleavage site. The histidine residues facilitate
30   purification by IMAC (immobilized metal ion affinity chromatography, as described in Porath *et al.*, *Prot. Exp. Purif.* 3, 263-281, 1992), while the enterokinase cleavage

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site provides a means for purifying the RTA-like-GPCR polypeptide from the fusion protein. Vectors which contain fusion proteins are disclosed in Kroll *et al.*, *DNA Cell Biol. 12*, 441-453, 1993.

5   *Chemical Synthesis*

Sequences encoding an RTA-like-GPCR polypeptide can be synthesized, in whole or in part, using chemical methods well known in the art (see Caruthers *et al.*, *Nucl. Acids Res. Symp. Ser.* 215-223, 1980; Horn *et al. Nucl. Acids Res. Symp. Ser.* 225-

10   232, 1980). Alternatively, an RTA-like-GPCR polypeptide itself can be produced using chemical methods to synthesize its amino acid sequence, such as by direct peptide synthesis using solid-phase techniques (Merrifield, *J. Am. Chem. Soc. 85*, 2149-2154, 1963; Roberge *et al.*, *Science 269*, 202-204, 1995). Protein synthesis can be performed using manual techniques or by automation. Automated synthesis can

15   be achieved, for example, using Applied Biosystems 431A Peptide Synthesizer (Perkin Elmer). Optionally, fragments of RTA-like-GPCR polypeptides can be separately synthesized and combined using chemical methods to produce a full-length molecule.

20   The newly synthesized peptide can be substantially purified by preparative high performance liquid chromatography (*e.g.*, Creighton, PROTEINS: STRUCTURES AND MOLECULAR PRINCIPLES, WH. Freeman and Co., New York, N.Y., 1983). The composition of a synthetic RTA-like-GPCR polypeptide can be confirmed by amino acid analysis or sequencing (*e.g.*, the Edman degradation procedure; *see* Creighton,

25   *supra*). Additionally, any portion of the amino acid sequence of the RTA-like-GPCR polypeptide can be altered during direct synthesis and/or combined using chemical methods with sequences from other proteins to produce a variant polypeptide or a fusion protein.

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Production of Altered RTA-like-GPCR Polypeptides

As will be understood by those of skill in the art, it may be advantageous to produce RTA-like-GPCR polypeptide-encoding nucleotide sequences possessing non-
5   naturally occurring codons. For example, codons preferred by a particular pro-karyotic or eukaryotic host can be selected to increase the rate of protein expression or to produce an RNA transcript having desirable properties, such as a half-life which is longer than that of a transcript generated from the naturally occurring sequence.

10   The nucleotide sequence disclosed herein can be engineered using methods generally known in the art to alter RTA-like-GPCR polypeptide-encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the polypeptide or mRNA product. DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic
15   oligonucleotides can be used to engineer the nucleotide sequences. For example, site-directed mutagenesis can be used to insert new restriction sites, alter glycosy-lation patterns, change codon preference, produce splice variants, introduce mutations, and so forth.

20   _Antibodies_

Any type of antibody known in the art can be generated to bind specifically to an epitope of an RTA-like-GPCR polypeptide. "Antibody" as used herein includes intact immunoglobulin molecules, as well as fragments thereof, such as Fab, F(ab')$_2$,
25   and Fv, which are capable of binding an epitope of an RTA-like-GPCR polypeptide. Typically, at least 6, 8, 10, or 12 contiguous amino acids are required to form an epitope. However, epitopes which involve non-contiguous amino acids may require more, e.g., at least 15, 25, or 50 amino acids.

30   An antibody which specifically binds to an epitope of an RTA-like-GPCR poly-peptide can be used therapeutically, as well as in immunochemical assays, such as

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Western blots, ELISAs, radioimmunoassays, immunohistochemical assays, immuno-
precipitations, or other immunochemical assays known in the art. Various immuno-
assays can be used to identify antibodies having the desired specificity. Numerous
protocols for competitive binding or immunoradiometric assays are well known in

5   the art. Such immunoassays typically involve the measurement of complex
formation between an immunogen and an antibody which specifically binds to the
immunogen.

Typically, an antibody which specifically binds to an RTA-like-GPCR polypeptide

10   provides a detection signal at least 5-, 10-, or 20-fold higher than a detection signal
provided with other proteins when used in an immunochemical assay. Preferably,
antibodies which specifically bind to RTA-like-GPCR polypeptides do not detect
other proteins in immunochemical assays and can immunoprecipitate an RTA-like-
GPCR polypeptide from solution.

15

RTA-like-GPCR polypeptides can be used to immunize a mammal, such as a mouse,
rat, rabbit, guinea pig, monkey, or human, to produce polyclonal antibodies. If
desired, an RTA-like-GPCR polypeptide can be conjugated to a carrier protein, such
as bovine serum albumin, thyroglobulin, and keyhole limpet hemocyanin.

20   Depending on the host species, various adjuvants can be used to increase the
immunological response. Such adjuvants include, but are not limited to, Freund's
adjuvant, mineral gels (e.g., aluminum hydroxide), and surface active substances
(e.g. lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole
limpet hemocyanin, and dinitrophenol). Among adjuvants used in humans, BCG

25   (bacilli Calmette-Guerin) and Corynebacterium parvum are especially useful.

Monoclonal antibodies which specifically bind to an RTA-like-GPCR polypeptide
can be prepared using any technique which provides for the production of antibody
molecules by continuous cell lines in culture. These techniques include, but are not

30   limited to, the hybridoma technique, the human B-cell hybridoma technique, and the
EBV-hybridoma technique (Kohler et al., Nature 256, 495-497, 1985; Kozbor et al.,

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J. Immunol. Methods 81, 31-42, 1985; Cote *et al., Proc. Natl. Acad. Sci. 80*, 2026-2030, 1983; Cole *et al., Mol. Cell Biol. 62*, 109-120, 1984).

5

In addition, techniques developed for the production of "chimeric antibodies," the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity, can be used (Morrison *et al., Proc. Natl. Acad. Sci. 81*, 6851-6855, 1984; Neuberger *et al., Nature 312*, 604-608, 1984; Takeda *et al., Nature 314*, 452-454, 1985). Monoclonal and other antibodies also can be "humanized" to prevent a patient from mounting an immune response

10 against the antibody when it is used therapeutically. Such antibodies may be sufficiently similar in sequence to human antibodies to be used directly in therapy or may require alteration of a few key residues. Sequence differences between rodent antibodies and human sequences can be minimized by replacing residues which differ from those in the human sequences by site directed mutagenesis of individual

15 residues or by grating of entire complementarity determining regions. Alternatively, humanized antibodies can be produced using recombinant methods, as described in GB2188638B. Antibodies which specifically bind to an RTA-like-GPCR poly-peptide can contain antigen binding sites which are either partially or fully humanized, as disclosed in U.S. 5,565,332.

20

Alternatively, techniques described for the production of single chain antibodies can be adapted using methods known in the art to produce single chain antibodies which specifically bind to RTA-like-GPCR polypeptides. Antibodies with related speci-ficity, but of distinct idiotypic composition, can be generated by chain shuffling from

25 random combinatorial immunoglobin libraries (Burton, *Proc. Natl. Acad. Sci. 88*, 11120-23, 1991).

Single-chain antibodies also can be constructed using a DNA amplification method, such as PCR, using hybridoma cDNA as a template (Thirion *et al.*, 1996, *Eur. J.*

30 *Cancer Prev. 5*, 507-11). Single-chain antibodies can be mono- or bispecific, and can be bivalent or tetravalent. Construction of tetravalent, bispecific single-chain

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antibodies is taught, for example, in Coloma & Morrison, 1997, *Nat. Biotechnol. 15*, 159-63. Construction of bivalent, bispecific single-chain antibodies is taught in Mallender & Voss, 1994, *J. Biol. Chem. 269*, 199-206.

5   A nucleotide sequence encoding a single-chain antibody can be constructed using manual or automated nucleotide synthesis, cloned into an expression construct using standard recombinant DNA methods, and introduced into a cell to express the coding sequence, as described below. Alternatively, single-chain antibodies can be produced directly using, for example, filamentous phage technology (Verhaar *et al.*,
10   1995, *Int. J. Cancer 61*, 497-501; Nicholls *et al.*, 1993, *J. Immunol. Meth. 165*, 81-91).

Antibodies which specifically bind to RTA-like-GPCR polypeptides also can be produced by inducing *in vivo* production in the lymphocyte population or by
15   screening immunoglobulin libraries or panels of highly specific binding reagents as disclosed in the literature (Orlandi *et al.*, *Proc. Natl. Acad. Sci. 86*, 3833-3837, 1989; Winter *et al.*, *Nature 349*, 293-299, 1991).

Other types of antibodies can be constructed and used therapeutically in methods of
20   the invention. For example, chimeric antibodies can be constructed as disclosed in WO 93/03151. Binding proteins which are derived from immunoglobulins and which are multivalent and multispecific, such as the "diabodies" described in WO 94/13804, also can be prepared.

25   Antibodies according to the invention can be purified by methods well known in the art. For example, antibodies can be affinity purified by passage over a column to which an RTA-like-GPCR polypeptide is bound. The bound antibodies can then be eluted from the column using a buffer with a high salt concentration.

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Antisense Oligonucleotides

Antisense oligonucleotides are nucleotide sequences which are complementary to a
specific DNA or RNA sequence. Once introduced into a cell, the complementary

5   nucleotides combine with natural sequences produced by the cell to form complexes
and block either transcription or translation. Preferably, an antisense oligonucleotide
is at least 11 nucleotides in length, but can be at least 12, 15, 20, 25, 30, 35, 40, 45,
or 50 or more nucleotides long. Longer sequences also can be used. Antisense
oligonucleotide molecules can be provided in a DNA construct and introduced into a

10   cell as described above to decrease the level of RTA-like-GPCR gene products in the
cell.

Antisense oligonucleotides can be deoxyribonucleotides, ribonucleotides, or a com-
bination of both. Oligonucleotides can be synthesized manually or by an automated

15   synthesizer, by covalently linking the 5' end of one nucleotide with the 3' end of
another nucleotide with non-phosphodiester internucleotide linkages such alkyl-
phosphonates, phosphorothioates, phosphorodithioates, alkylphosphonothioates,
alkylphosphonates, phosphoramidates, phosphate esters, carbamates, acetamidate,
carboxymethyl esters, carbonates, and phosphate triesters. *See* Brown, *Meth. Mol.*

20   *Biol. 20*, 1-8, 1994; Sonveaux, *Meth. Mol. Biol. 26*, 1-72, 1994; Uhlmann *et al.*,
Chem. Rev. 90, 543-583, 1990.

Modifications of RTA-like-GPCR gene expression can be obtained by designing
antisense oligonucleotides which will form duplexes to the control, 5', or regulatory

25   regions of the RTA-like-GPCR gene. Oligonucleotides derived from the tran-
scription initiation site, *e.g.*, between positions -10 and +10 from the start site, are
preferred. Similarly, inhibition can be achieved using "triple helix" base-pairing
methodology. Triple helix pairing is useful because it causes inhibition of the ability
of the double helix to open sufficiently for the binding of polymerases, transcription

30   factors, or chaperons. Therapeutic advances using triplex DNA have been described
in the literature (*e.g.*, Gee *et al.*, in Huber & Carr, MOLECULAR AND IMMUNOLOGIC

APPROACHES, Futura Publishing Co., Mt. Kisco, N.Y., 1994). An antisense oligonucleotide also can be designed to block translation of mRNA by preventing the transcript from binding to ribosomes.

5   Precise complementarity is not required for successful complex formation between an antisense oligonucleotide and the complementary sequence of an RTA-like-GPCR polynucleotide. Antisense oligonucleotides which comprise, for example, 2, 3, 4, or 5 or more stretches of contiguous nucleotides which are precisely complementary to an RTA-like-GPCR polynucleotide, each separated by a stretch of contiguous

10   nucleotides which are not complementary to adjacent RTA-like-GPCR nucleotides, can provide sufficient targeting specificity for RTA-like-GPCR mRNA. Preferably, each stretch of complementary contiguous nucleotides is at least 4, 5, 6, 7, or 8 or more nucleotides in length. Non-complementary intervening sequences are preferably 1, 2, 3, or 4 nucleotides in length. One skilled in the art can easily use the

15   calculated melting point of an antisense-sense pair to determine the degree of mismatching which will be tolerated between a particular antisense oligonucleotide and a particular RTA-like-GPCR polynucleotide sequence.

Antisense oligonucleotides can be modified without affecting their ability to

20   hybridize to an RTA-like-GPCR polynucleotide. These modifications can be internal or at one or both ends of the antisense molecule. For example, internucleoside phosphate linkages can be modified by adding cholesteryl or diamine moieties with varying numbers of carbon residues between the amino groups and terminal ribose. Modified bases and/or sugars, such as arabinose instead of ribose, or a 3', 5'-

25   substituted oligonucleotide in which the 3' hydroxyl group or the 5' phosphate group are substituted, also can be employed in a modified antisense oligonucleotide. These modified oligonucleotides can be prepared by methods well known in the art. *See, e.g.*, Agrawal *et al.*, *Trends Biotechnol. 10*, 152-158, 1992; Uhlmann *et al.*, *Chem. Rev. 90*, 543-584, 1990; Uhlmann *et al.*, *Tetrahedron. Lett. 215*, 3539-3542, 1987.

30

Ribozymes

Ribozymes are RNA molecules with catalytic activity. *See, e.g.,* Cech, *Science 236,* 1532-1539; 1987; Cech, *Ann. Rev. Biochem. 59,* 543-568; 1990, Cech, *Curr. Opin. Struct. Biol. 2,* 605-609; 1992, Couture & Stinchcomb, *Trends Genet. 12,* 510-515, 1996. Ribozymes can be used to inhibit gene function by cleaving an RNA sequence, as is known in the art (*e.g.,* Haseloff *et al.,* U.S. Patent 5,641,673). The mechanism of ribozyme action involves sequence-specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. Examples include engineered hammerhead motif ribozyme molecules that can specifically and efficiently catalyze endonucleolytic cleavage of specific nucleotide sequences.

A coding sequence of an RTA-like-GPCR polynucleotide can be used to generate ribozymes which will specifically bind to mRNA transcribed from the RTA-like-GPCR polynucleotide. Methods of designing and constructing ribozymes which can cleave other RNA molecules in trans in a highly sequence specific manner have been developed and described in the art (*see* Haseloff *et al. Nature 334,* 585-591, 1988). For example, the cleavage activity of ribozymes can be targeted to specific RNAs by engineering a discrete "hybridization" region into the ribozyme. The hybridization region contains a sequence complementary to the target RNA and thus specifically hybridizes with the target (see, for example, Gerlach *et al.,* EP 321,201).

Specific ribozyme cleavage sites within an RTA-like-GPCR RNA target can be identified by scanning the target molecule for ribozyme cleavage sites which include the following sequences: GUA, GUU, and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides corresponding to the region of the target RNA containing the cleavage site can be evaluated for secondary structural features which may render the target inoperable. Suitability of candidate RTA-like-GPCR RNA targets also can be evaluated by testing accessibility to hybridization with complementary oligonucleotides using ribonuclease protection assays. The

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nucleotide sequence shown in SEQ ID NO:1 and its complement provide sources of suitable hybridization region sequences. Longer complementary sequences can be used to increase the affinity of the hybridization sequence for the target. The hybridizing and cleavage regions of the ribozyme can be integrally related such that
5   upon hybridizing to the target RNA through the complementary regions, the catalytic region of the ribozyme can cleave the target.

Ribozymes can be introduced into cells as part of a DNA construct. Mechanical methods, such as microinjection, liposome-mediated transfection, electroporation, or
10 calcium phosphate precipitation, can be used to introduce a ribozyme-containing DNA construct into cells in which it is desired to decrease RTA-like-GPCR expression. Alternatively, if it is desired that the cells stably retain the DNA construct, the construct can be supplied on a plasmid and maintained as a separate element or integrated into the genome of the cells, as is known in the art. A
15 ribozyme-encoding DNA construct can include transcriptional regulatory elements, such as a promoter element, an enhancer or UAS element, and a transcriptional terminator signal, for controlling transcription of ribozymes in the cells.

As taught in Haseloff *et al.*, U.S. Patent 5,641,673, ribozymes can be engineered so
20 that ribozyme expression will occur in response to factors which induce expression of a target gene. Ribozymes also can be engineered to provide an additional level of regulation, so that destruction of mRNA occurs only when both a ribozyme and a target gene are induced in the cells.

25 *Screening Methods*

The invention provides assays for screening test compounds which bind to or modulate the activity of an RTA-like-GPCR polypeptide or an RTA-like-GPCR polynucleotide. A test compound preferably binds to an RTA-like-GPCR poly-
30 peptide or polynucleotide. More preferably, a test compound decreases or increases a biological effect mediated via human RTA-like-GPCR by at least about 10,

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preferably about 50, more preferably about 75, 90, or 100% relative to the absence of the test compound.

Test Compounds

Test compounds can be pharmacologic agents already known in the art or can be compounds previously unknown to have any pharmacological activity. The compounds can be naturally occurring or designed in the laboratory. They can be isolated from microorganisms, animals, or plants, and can be produced recombinantly, or synthesized by chemical methods known in the art. If desired, test compounds can be obtained using any of the numerous combinatorial library methods known in the art, including but not limited to, biological libraries, spatially addressable parallel solid phase or solution phase libraries, synthetic library methods requiring deconvolution, the "one-bead one-compound" library method, and synthetic library methods using affinity chromatography selection. The biological library approach is limited to polypeptide libraries, while the other four approaches are applicable to polypeptide, non-peptide oligomer, or small molecule libraries of compounds. *See* Lam, *Anticancer Drug Des. 12*, 145, 1997.

Methods for the synthesis of molecular libraries are well known in the art (*see*, for example, DeWitt *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 90*, 6909, 1993; Erb *et al. Proc. Natl. Acad. Sci. U.S.A. 91*, 11422, 1994; Zuckermann *et al.*, *J. Med. Chem. 37*, 2678, 1994; Cho *et al.*, *Science 261*, 1303, 1993; Carell *et al.*, *Angew. Chem. Int. Ed. Engl. 33*, 2059, 1994; Carell *et al.*, *Angew. Chem. Int. Ed. Engl. 33*, 2061; Gallop *et al.*, *J. Med. Chem. 37*, 1233, 1994). Libraries of compounds can be presented in solution (*see, e.g.*, Houghten, *Biotechniques 13*, 412-421, 1992), or on beads (Lam, *Nature 354*, 82-84, 1991), chips (Fodor, *Nature 364*, 555-556, 1993), bacteria or spores (Ladner, U.S. Patent 5,223,409), plasmids (Cull *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 89*, 1865-1869, 1992), or phage (Scott & Smith, *Science 249*, 386-390, 1990; Devlin, *Science 249*, 404-406, 1990); Cwirla *et al.*, *Proc. Natl. Acad. Sci. 97*, 6378-6382, 1990; Felici, *J. Mol. Biol. 222*, 301-310, 1991; and Ladner, U.S. Patent 5,223,409).

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High Throughput Screening

Test compounds can be screened for the ability to bind to RTA-like-GPCR polypeptides or polynucleotides or to affect RTA-like-GPCR activity or RTA-like-GPCR gene expression using high throughput screening. Using high throughput screening, many discrete compounds can be tested in parallel so that large numbers of test compounds can be quickly screened. The most widely established techniques utilize 96-well microtiter plates. The wells of the microtiter plates typically require assay volumes that range from 50 to 500 μl. In addition to the plates, many instruments, materials, pipettors, robotics, plate washers, and plate readers are commercially available to fit the 96-well format.

Alternatively, "free format assays," or assays that have no physical barrier between samples, can be used. For example, an assay using pigment cells (melanocytes) in a simple homogeneous assay for combinatorial peptide libraries is described by Jayawickreme et al., Proc. Natl. Acad. Sci. U.S.A. 19, 1614-18 (1994). The cells are placed under agarose in petri dishes, then beads that carry combinatorial compounds are placed on the surface of the agarose. The combinatorial compounds are partially released the compounds from the beads. Active compounds can be visualized as dark pigment areas because, as the compounds diffuse locally into the gel matrix, the active compounds cause the cells to change colors.

Another example of a free format assay is described by Chelsky, "Strategies for Screening Combinatorial Libraries: Novel and Traditional Approaches," reported at the First Annual Conference of The Society for Biomolecular Screening in Philadelphia, Pa. (Nov. 7-10, 1995). Chelsky placed a simple homogenous enzyme assay for carbonic anhydrase inside an agarose gel such that the enzyme in the gel would cause a color change throughout the gel. Thereafter, beads carrying combinatorial compounds via a photolinker were placed inside the gel and the

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compounds were partially released by UV-light. Compounds that inhibited the enzyme were observed as local zones of inhibition having less color change.

Yet another example is described by Salmon *et al.*, *Molecular Diversity 2*, 57-63 (1996). In this example, combinatorial libraries were screened for compounds that had cytotoxic effects on cancer cells growing in agar.

Another high throughput screening method is described in Beutel *et al.*, U.S. Patent 5,976,813. In this method, test samples are placed in a porous matrix. One or more assay components are then placed within, on top of, or at the bottom of a matrix such as a gel, a plastic sheet, a filter, or other form of easily manipulated solid support. When samples are introduced to the porous matrix they diffuse sufficiently slowly, such that the assays can be performed without the test samples running together.

Binding Assays

For binding assays, the test compound is preferably a small molecule which binds to and occupies the active site of the RTA-like-GPCR polypeptide, thereby making the ligand binding site inaccessible to substrate such that normal biological activity is prevented. Examples of such small molecules include, but are not limited to, small peptides or peptide-like molecules. Potential ligands which bind to a polypeptide of the invention include, but are not limited to, the natural ligands of known RTA-like-GPCRs and analogues or derivatives thereof. Natural ligands of GPCRs include adrenomedullin, amylin, calcitonin gene related protein (CGRP), calcitonin, anandamide, serotonin, histamine, adrenalin, noradrenalin, platelet activating factor, thrombin, C5a, bradykinin, and chemokines.

In binding assays, either the test compound or the RTA-like-GPCR polypeptide can comprise a detectable label, such as a fluorescent, radioisotopic, chemiluminescent, or enzymatic label, such as horseradish peroxidase, alkaline phosphatase, or luciferase. Detection of a test compound which is bound to the RTA-like-GPCR

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polypeptide can then be accomplished, for example, by direct counting of radioemmission, by scintillation counting, or by determining conversion of an appropriate substrate to a detectable product.

5   Alternatively, binding of a test compound to an RTA-like-GPCR polypeptide can be determined without labeling either of the interactants. For example, a micro-physiometer can be used to detect binding of a test compound with an RTA-like-GPCR polypeptide. A microphysiometer (*e.g.*, Cytosensor™) is an analytical instrument that measures the rate at which a cell acidifies its environment using a
10   light-addressable potentiometric sensor (LAPS). Changes in this acidification rate can be used as an indicator of the interaction between a test compound and an RTA-like-GPCR polypeptide (McConnell *et al.*, *Science 257*, 1906-1912, 1992).

Determining the ability of a test compound to bind to an RTA-like-GPCR poly-
15   peptide also can be accomplished using a technology such as real-time Bimolecular Interaction Analysis (BIA) (Sjolander & Urbaniczky, *Anal. Chem. 63*, 2338-2345, 1991, and Szabo *et al.*, *Curr. Opin. Struct. Biol. 5*, 699-705, 1995). BIA is a techno-logy for studying biospecific interactions in real time, without labeling any of the interactants (*e.g.*, BIAcore™). Changes in the optical phenomenon surface plasmon
20   resonance (SPR) can be used as an indication of real-time reactions between biological molecules.

In yet another aspect of the invention, an RTA-like-GPCR polypeptide can be used as a "bait protein" in a two-hybrid assay or three-hybrid assay (see, *e.g.*, U.S. Patent
25   5,283,317; Zervos *et al.*, *Cell 72*, 223-232, 1993; Madura *et al.*, *J. Biol. Chem. 268*, 12046-12054, 1993; Bartel *et al.*, *Biotechniques 14*, 920-924, 1993; Iwabuchi *et al.*, *Oncogene 8*, 1693-1696, 1993; and Brent WO94/10300), to identify other proteins which bind to or interact with the RTA-like-GPCR polypeptide and modulate its activity.

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The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. For example, in one construct, polynucleotide encoding an RTA-like-GPCR polypeptide can be fused to a polynucleotide encoding

5   the DNA binding domain of a known transcription factor (e.g., GAL-4). In the other construct a DNA sequence that encodes an unidentified protein ("prey" or "sample") can be fused to a polynucleotide that codes for the activation domain of the known transcription factor. If the "bait" and the "prey" proteins are able to interact in vivo to form an protein-dependent complex, the DNA-binding and activation domains of the

10   transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (e.g., LacZ), which is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected, and cell colonies containing the functional transcription factor can be isolated and used to obtain the DNA sequence encoding

15   the protein which interacts with the RTA-like-GPCR polypeptide.

It may be desirable to immobilize either the RTA-like-GPCR polypeptide (or polynucleotide) or the test compound to facilitate separation of bound from unbound forms of one or both of the interactants, as well as to accommodate automation of the

20   assay. Thus, either the RTA-like-GPCR polypeptide (or polynucleotide) or the test compound can be bound to a solid support. Suitable solid supports include, but are not limited to, glass or plastic slides, tissue culture plates, microtiter wells, tubes, silicon chips, or particles such as beads (including, but not limited to, latex, polystyrene, or glass beads). Any method known in the art can be used to attach the

25   RTA-like-GPCR polypeptide (or polynucleotide) or test compound to a solid support, including use of covalent and non-covalent linkages, passive absorption, or pairs of binding moieties attached respectively to the polypeptide (or polynucleotide) or test compound and the solid support. Test compounds are preferably bound to the solid support in an array, so that the location of individual test compounds can be

30   tracked. Binding of a test compound to an RTA-like-GPCR polypeptide (or poly-nucleotide) can be accomplished in any vessel suitable for containing the reactants.

Examples of such vessels include microtiter plates, test tubes, and microcentrifuge tubes.

In one embodiment, the RTA-like-GPCR polypeptide is a fusion protein comprising a domain that allows the RTA-like-GPCR polypeptide to be bound to a solid support. For example, glutathione-S-transferase fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, Mo.) or glutathione derivatized microtiter plates, which are then combined with the test compound or the test compound and the non-adsorbed RTA-like-GPCR polypeptide; the mixture is then incubated under conditions conducive to complex formation (*e.g.*, at physiological conditions for salt and pH). Following incubation, the beads or microtiter plate wells are washed to remove any unbound components. Binding of the interactants can be determined either directly or indirectly, as described above. Alternatively, the complexes can be dissociated from the solid support before binding is determined.

Other techniques for immobilizing proteins or polynucleotides on a solid support also can be used in the screening assays of the invention. For example, either an RTA-like-GPCR polypeptide (or polynucleotide) or a test compound can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated RTA-like-GPCR poly-peptides (or polynucleotides) or test compounds can be prepared from biotin-NHS-(N-hydroxysuccinimide) using techniques well known in the art (*e.g.*, biotinylation kit, Pierce Chemicals, Rockford, Ill.) and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, antibodies which specifically bind to an RTA-like-GPCR polypeptide, polynucleotide, or a test compound, but which do not interfere with a desired binding site, such as the active site of the RTA-like-GPCR polypeptide, can be derivatized to the wells of the plate. Unbound target or protein can be trapped in the wells by antibody conjugation.

Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using anti-

bodies which specifically bind to the RTA-like-GPCR polypeptide or test compound, enzyme-linked assays which rely on detecting an activity of the RTA-like-GPCR polypeptide, and SDS gel electrophoresis under non-reducing conditions.

5   Screening for test compounds which bind to an RTA-like-GPCR polypeptide or polynucleotide also can be carried out in an intact cell. Any cell which comprises an RTA-like-GPCR polypeptide or polynucleotide can be used in a cell-based assay system. An RTA-like-GPCR polynucleotide can be naturally occurring in the cell or can be introduced using techniques such as those described above. Binding of the
10   test compound to an RTA-like-GPCR polypeptide or polynucleotide is determined as described above.

Functional Assays

15   Test compounds can be tested for the ability to increase or decrease a biological effect of an RTA-like-GPCR polypeptide. Such biological effects can be determined using the functional assays described in the specific examples, below. Functional assays can be carried out after contacting either a purified RTA-like-GPCR polypeptide, a cell membrane preparation, or an intact cell with a test compound. A
20   test compound which decreases a functional activity of an RTA-like-GPCR by at least about 10, preferably about 50, more preferably about 75, 90, or 100% is identified as a potential agent for decreasing RTA-like-GPCR activity. A test compound which increases RTA-like-GPCR activity by at least about 10, preferably about 50, more preferably about 75, 90, or 100% is identified as a potential agent for
25   increasing RTA-like-GPCR activity.

One such screening procedure involves the use of melanophores which are transfected to express an RTA-like-GPCR polypeptide. Such a screening technique is described in WO 92/01810 published Feb. 6, 1992. Thus, for example, such an
30   assay may be employed for screening for a compound which inhibits activation of the receptor polypeptide by contacting the melanophore cells which comprise the

receptor with both the receptor ligand and a test compound to be screened. Inhibition of the signal generated by the ligand indicates that a test compound is a potential antagonist for the receptor, *i.e.*, inhibits activation of the receptor. The screen may be employed for identifying a test compound which activates the receptor by contacting

5   such cells with compounds to be screened and determining whether each test compound generates a signal, *i.e.*, activates the receptor.

Other screening techniques include the use of cells which express a human RTA-like-GPCR polypeptide (for example, transfected CHO cells) in a system which

10   measures extracellular pH changes caused by receptor activation (*see, e.g., Science 246*, 181-296, 1989). For example, test compounds may be contacted with a cell which expresses a human RTA-like-GPCR polypeptide and a second messenger response, *e.g.*, signal transduction or pH changes, can be measured to determine whether the test compound activates or inhibits the receptor.

15

Another such screening technique involves introducing RNA encoding a human RTA-like-GPCR polypeptide into *Xenopus* oocytes to transiently express the receptor. The transfected oocytes can then be contacted with the receptor ligand and a test compound to be screened, followed by detection of inhibition or activation of a

20   calcium signal in the case of screening for test compounds which are thought to inhibit activation of the receptor.

Another screening technique involves expressing a human RTA-like-GPCR polypeptide in cells in which the receptor is linked to a phospholipase C or D. Such

25   cells include endothelial cells, smooth muscle cells, embryonic kidney cells, etc. The screening may be accomplished as described above by quantifying the degree of activation of the receptor from changes in the phospholipase activity.

Details of functional assays such as those described above are provided in the

30   specific examples, below.

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RTA-like-GPCR Gene Expression

In another embodiment, test compounds which increase or decrease RTA-like-GPCR gene expression are identified. An RTA-like-GPCR polynucleotide is contacted with a test compound, and the expression of an RNA or polypeptide product of the RTA-like-GPCR polynucleotide is determined. The level of expression of appropriate mRNA or polypeptide in the presence of the test compound is compared to the level of expression of mRNA or polypeptide in the absence of the test compound. The test compound can then be identified as a modulator of expression based on this comparison. For example, when expression of mRNA or polypeptide is greater in the presence of the test compound than in its absence, the test compound is identified as a stimulator or enhancer of the mRNA or polypeptide expression. Alternatively, when expression of the mRNA or polypeptide is less in the presence of the test compound than in its absence, the test compound is identified as an inhibitor of the mRNA or polypeptide expression.

The level of RTA-like-GPCR mRNA or polypeptide expression in the cells can be determined by methods well known in the art for detecting mRNA or polypeptide. Either qualitative or quantitative methods can be used. The presence of polypeptide products of an RTA-like-GPCR polynucleotide can be determined, for example, using a variety of techniques known in the art, including immunochemical methods such as radioimmunoassay, Western blotting, and immunohistochemistry. Alternatively, polypeptide synthesis can be determined _in vivo_, in a cell culture, or in an _in vitro_ translation system by detecting incorporation of labeled amino acids into an RTA-like-GPCR polypeptide.

Such screening can be carried out either in a cell-free assay system or in an intact cell. Any cell which expresses an RTA-like-GPCR polynucleotide can be used in a cell-based assay system. The RTA-like-GPCR polynucleotide can be naturally occurring in the cell or can be introduced using techniques such as those described

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above. Either a primary culture or an established cell line, such as CHO or human embryonic kidney 293 cells, can be used.

Pharmaceutical Compositions

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The invention also provides pharmaceutical compositions which can be administered to a patient to achieve a therapeutic effect. Pharmaceutical compositions of the invention can comprise, for example, an RTA-like-GPCR polypeptide, RTA-like-GPCR polynucleotide, antibodies which specifically bind to an RTA-like-GPCR

10   polypeptide, or mimetics, agonists, antagonists, or inhibitors of an RTA-like-GPCR polypeptide activity. The compositions can be administered alone or in combination with at least one other agent, such as stabilizing compound, which can be administered in any sterile, biocompatible pharmaceutical carrier, including, but not limited to, saline, buffered saline, dextrose, and water. The compositions can be

15   administered to a patient alone, or in combination with other agents, drugs or hormones.

In addition to the active ingredients, these pharmaceutical compositions can contain suitable pharmaceutically-acceptable carriers comprising excipients and auxiliaries

20   which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Pharmaceutical compositions of the invention can be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, intranasal, parenteral, topical, sublingual, or rectal

25   means. Pharmaceutical compositions for oral administration can be formulated using pharmaceutically acceptable carriers well known in the art in dosages suitable for oral administration. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for ingestion by the patient.

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Pharmaceutical preparations for oral use can be obtained through combination of active compounds with solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are carbohydrate or protein fillers,

5   such as sugars, including lactose, sucrose, mannitol, or sorbitol; starch from corn, wheat, rice, potato, or other plants; cellulose, such as methyl cellulose, hydroxy-propylmethyl-cellulose, or sodium carboxymethylcellulose; gums including arabic and tragacanth; and proteins such as gelatin and collagen. If desired, disintegrating or solubilizing agents can be added, such as the cross-linked polyvinyl pyrrolidone,

10   agar, alginic acid, or a salt thereof, such as sodium alginate.

Dragee cores can be used in conjunction with suitable coatings, such as concentrated sugar solutions, which also can contain gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and

15   suitable organic solvents or solvent mixtures. Dyestuffs or pigments can be added to the tablets or dragee coatings for product identification or to characterize the quantity of active compound, i.e., dosage.

Pharmaceutical preparations which can be used orally include push-fit capsules made

20   of gelatin, as well as soft, sealed capsules made of gelatin and a coating, such as glycerol or sorbitol. Push-fit capsules can contain active ingredients mixed with a filler or binders, such as lactose or starches, lubricants, such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the active compounds can be dissolved or suspended in suitable liquids, such as fatty oils, liquid, or liquid

25   polyethylene glycol with or without stabilizers.

Pharmaceutical formulations suitable for parenteral administration can be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks' solution, Ringer's solution, or physiologically buffered saline. Aqueous injection

30   suspensions can contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Additionally, sus-

pensions of the active compounds can be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Non-lipid polycationic amino polymers also can be used for delivery. Optionally, the

5   suspension also can contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions. For topical or nasal administration, penetrants appropriate to the particular barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

10

The pharmaceutical compositions of the present invention can be manufactured in a manner that is known in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping, or lyophilizing processes. The pharmaceutical composition can be provided as a salt

15   and can be formed with many acids, including but not limited to, hydrochloric, sulfuric, acetic, lactic, tartaric, malic, succinic, etc. Salts tend to be more soluble in aqueous or other protonic solvents than are the corresponding free base forms. In other cases, the preferred preparation can be a lyophilized powder which can contain any or all of the following: 1-50 mM histidine, 0.1%-2% sucrose, and 2-7%

20   mannitol, at a pH range of 4.5 to 5.5, that is combined with buffer prior to use.

Further details on techniques for formulation and administration can be found in the latest edition of REMINGTON'S PHARMACEUTICAL SCIENCES (Maack Publishing Co., Easton, Pa.). After pharmaceutical compositions have been prepared, they can be

25   placed in an appropriate container and labeled for treatment of an indicated condition. Such labeling would include amount, frequency, and method of administration.

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Therapeutic Indications and Methods

GPCRs are ubiquitous in the mammalian host and are responsible for many biological functions, including many pathologies. Accordingly, it is desirable to find

5   compounds and drugs which stimulate a GPCR on the one hand and which can inhibit the function of a GPCR on the other hand. For example, compounds which activate a GPCR may be employed for therapeutic purposes, such as the treatment of asthma, Parkinson's disease, acute heart failure, urinary retention, and osteoporosis. In particular, compounds which activate GPCRs are useful in treating various

10   cardiovascular ailments such as caused by the lack of pulmonary blood flow or hypertension. In addition these compounds may also be used in treating various physiological disorders relating to abnormal control of fluid and electrolyte homeostasis and in diseases associated with abnormal angiotensin-induced aldosterone secretion.

15

In general, compounds which inhibit activation of a GPCR can be used for a variety of therapeutic purposes, for example, for the treatment of hypotension and/or hypertension, angina pectoris, myocardial infarction, ulcers, asthma, allergies, benign prostatic hypertrophy, and psychotic and neurological disorders including

20   schizophrenia, manic excitement, depression, delirium, dementia or severe mental retardation, dyskinesias, such as Huntington's disease or Tourett's syndrome, among others. Compounds which inhibit GPCRs also are useful in reversing endogenous anorexia, in the control of bulimia, and in treating various cardiovascular ailments such as caused by excessive pulmonary blood flow or hypotension.

25

This invention further pertains to the use of novel agents identified by the screening assays described above. Accordingly, it is within the scope of this invention to use a test compound identified as described herein in an appropriate animal model. For example, an agent identified as described herein (*e.g.*, a modulating agent, an

30   antisense nucleic acid molecule, a specific antibody, ribozyme, or an RTA-like-GPCR polypeptide binding molecule) can be used in an animal model to determine

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the efficacy, toxicity, or side effects of treatment with such an agent. Alternatively, an agent identified as described herein can be used in an animal model to determine the mechanism of action of such an agent. Furthermore, this invention pertains to uses of novel agents identified by the above-described screening assays for treatments as described herein.

A reagent which affects RTA-like-GPCR activity can be administered to a human cell, either *in vitro* or *in vivo*, to reduce RTA-like-GPCR activity. The reagent preferably binds to an expression product of a human RTA-like-GPCR gene. If the expression product is a protein, the reagent is preferably an antibody. For treatment of human cells *ex vivo*, an antibody can be added to a preparation of stem cells which have been removed from the body. The cells can then be replaced in the same or another human body, with or without clonal propagation, as is known in the art.

In one embodiment, the reagent is delivered using a liposome. Preferably, the liposome is stable in the animal into which it has been administered for at least about 30 minutes, more preferably for at least about 1 hour, and even more preferably for at least about 24 hours. A liposome comprises a lipid composition that is capable of targeting a reagent, particularly a polynucleotide, to a particular site in an animal, such as a human. Preferably, the lipid composition of the liposome is capable of targeting to a specific organ of an animal, such as the lung, liver, spleen, heart brain, lymph nodes, and skin.

A liposome useful in the present invention comprises a lipid composition that is capable of fusing with the plasma membrane of the targeted cell to deliver its contents to the cell. Preferably, the transfection efficiency of a liposome is about 0.5 μg of DNA per 16 nmole of liposome delivered to about 10^6 cells, more preferably about 1.0 μg of DNA per 16 nmole of liposome delivered to about 10^6 cells, and even more preferably about 2.0 μg of DNA per 16 nmol of liposome delivered to about 10^6 cells. Preferably, a liposome is between about 100 and 500 nm, more preferably

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between about 150 and 450 nm, and even more preferably between about 200 and 400 nm in diameter.

5   Suitable liposomes for use in the present invention include those liposomes standardly used in, for example, gene delivery methods known to those of skill in the art. More preferred liposomes include liposomes having a polycationic lipid composition and/or liposomes having a cholesterol backbone conjugated to polyethylene glycol. Optionally, a liposome comprises a compound capable of targeting the liposome to a tumor cell, such as a tumor cell ligand exposed on the

10   outer surface of the liposome.

Complexing a liposome with a reagent such as an antisense oligonucleotide or ribozyme can be achieved using methods which are standard in the art (see, for example, U.S. Patent 5,705,151). Preferably, from about 0.1 µg to about 10 µg of

15   polynucleotide is combined with about 8 nmol of liposomes, more preferably from about 0.5 µg to about 5 µg of polynucleotides are combined with about 8 nmol liposomes, and even more preferably about 1.0 µg of polynucleotides is combined with about 8 nmol liposomes.

20   In another embodiment, antibodies can be delivered to specific tissues *in vivo* using receptor-mediated targeted delivery. Receptor-mediated DNA delivery techniques are taught in, for example, Findeis *et al. Trends in Biotechnol. 11*, 202-05 (1993); Chiou *et al.*, GENE THERAPEUTICS: METHODS AND APPLICATIONS OF DIRECT GENE TRANSFER (J.A. Wolff, ed.) (1994); Wu & Wu, *J. Biol. Chem. 263*, 621-24 (1988);

25   Wu *et al.*, *J. Biol. Chem. 269*, 542-46 (1994); Zenke *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 87*, 3655-59 (1990); Wu *et al.*, *J. Biol. Chem. 266*, 338-42 (1991).

Determination of a Therapeutically Effective Dose

30   The determination of a therapeutically effective dose is well within the capability of those skilled in the art. A therapeutically effective dose refers to that amount of

active ingredient which increases or decreases RTA-like-GPCR activity relative to the RTA-like-GPCR activity which occurs in the absence of the therapeutically effective dose.

5   For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays or in animal models, usually mice, rabbits, dogs, or pigs. The animal model also can be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

10

Therapeutic efficacy and toxicity, *e.g.*, ED_{50} (the dose therapeutically effective in 50% of the population) and LD_{50} (the dose lethal to 50% of the population), can be determined by standard pharmaceutical procedures in cell cultures or experimental animals. The dose ratio of toxic to therapeutic effects is the therapeutic index, and it

15   can be expressed as the ratio, LD_{50}/ED_{50}.

Pharmaceutical compositions which exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies is used in formulating a range of dosage for human use. The dosage contained in such compositions is

20   preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage varies within this range depending upon the dosage form employed, sensitivity of the patient, and the route of administration.

The exact dosage will be determined by the practitioner, in light of factors related to

25   the subject that requires treatment. Dosage and administration are adjusted to provide sufficient levels of the active ingredient or to maintain the desired effect. Factors which can be taken into account include the severity of the disease state, general health of the subject, age, weight, and gender of the subject, diet, time and frequency of administration, drug combination(s), reaction sensitivities, and

30   tolerance/response to therapy. Long-acting pharmaceutical compositions can be

administered every 3 to 4 days, every week, or once every two weeks depending on the half-life and clearance rate of the particular formulation.

Normal dosage amounts can vary from 0.1 to 100,000 micrograms, up to a total dose
-5   of about 1 g, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature and generally available to practitioners in the art. Those skilled in the art will employ different formulations for nucleotides than for proteins or their inhibitors. Similarly, delivery of poly-nucleotides or polypeptides will be specific to particular cells, conditions, locations,
10   etc.

If the reagent is a single-chain antibody, polynucleotides encoding the antibody can be constructed and introduced into a cell either *ex vivo* or *in vivo* using well-established techniques including, but not limited to, transferrin-polycation-mediated
15   DNA transfer, transfection with naked or encapsulated nucleic acids, liposome-mediated cellular fusion, intracellular transportation of DNA-coated latex beads, protoplast fusion, viral infection, electroporation, "gene gun," and DEAE- or calcium phosphate-mediated transfection.

20   Effective *in vivo* dosages of an antibody are in the range of about 5 µg to about 50 µg/kg, about 50 µg to about 5 mg/kg, about 100 µg to about 500 µg/kg of patient body weight, and about 200 to about 250 µg/kg of patient body weight. For administration of polynucleotides encoding single-chain antibodies, effective *in vivo* dosages are in the range of about 100 ng to about 200 ng, 500 ng to about 50 mg,
25   about 1 µg to about 2 mg, about 5 µg to about 500 µg, and about 20 µg to about 100 µg of DNA.

If the expression product is mRNA, the reagent is preferably an antisense oligonucleotide or a ribozyme. Polynucleotides which express antisense oligo-
30   nucleotides or ribozymes can be introduced into cells by a variety of methods, as described above.

Preferably, a reagent reduces expression of an RTA-like-GPCR gene or the activity of an RTA-like-GPCR polypeptide by at least about 10, preferably about 50, more preferably about 75, 90, or 100% relative to the absence of the reagent. The effectiveness of the mechanism chosen to decrease the level of expression of an RTA-like-GPCR gene or the activity of an RTA-like-GPCR polypeptide can be assessed using methods well known in the art, such as hybridization of nucleotide probes to RTA-like-GPCR-specific mRNA, quantitative RT-PCR, immunologic detection of an RTA-like-GPCR polypeptide, or measurement of RTA-like-GPCR activity.

In any of the embodiments described above, any of the pharmaceutical compositions of the invention can be administered in combination with other appropriate therapeutic agents. Selection of the appropriate agents for use in combination therapy can be made by one of ordinary skill in the art, according to conventional pharmaceutical principles. The combination of therapeutic agents can act synergistically to effect the treatment or prevention of the various disorders described above. Using this approach, one may be able to achieve therapeutic efficacy with lower dosages of each agent, thus reducing the potential for adverse side effects.

Any of the therapeutic methods described above can be applied to any subject in need of such therapy, including, for example, mammals such as dogs, cats, cows, horses, rabbits, monkeys, and most preferably, humans.

Diagnostic Methods

GPCRs also can be used in diagnostic assays for detecting diseases and abnormalities or susceptibility to diseases and abnormalities related to the presence of mutations in the nucleic acid sequences which encode a GPCR. Such diseases, by way of example, are related to cell transformation, such as tumors and cancers, and various cardiovascular disorders, including hypertension and hypotension, as well as diseases

arising from abnormal blood flow, abnormal angiotensin-induced aldosterone secretion, and other abnormal control of fluid and electrolyte homeostasis.

Differences can be determined between the cDNA or genomic sequence encoding a
5   GPCR in individuals afflicted with a disease and in normal individuals. If a mutation is observed in some or all of the afflicted individuals but not in normal individuals, then the mutation is likely to be the causative agent of the disease.

Sequence differences between a reference gene and a gene having mutations can be
10   revealed by the direct DNA sequencing method. In addition, cloned DNA segments can be employed as probes to detect specific DNA segments. The sensitivity of this method is greatly enhanced when combined with PCR. For example, a sequencing primer can be used with a double-stranded PCR product or a single-stranded template molecule generated by a modified PCR. The sequence determination is performed
15   by conventional procedures using radiolabeled nucleotides or by automatic sequencing procedures using fluorescent tags.

Genetic testing based on DNA sequence differences can be carried out by detection of alteration in electrophoretic mobility of DNA fragments in gels with or without
20   denaturing agents. Small sequence deletions and insertions can be visualized, for example, by high resolution gel electrophoresis. DNA fragments of different sequences can be distinguished on denaturing formamide gradient gels in which the mobilities of different DNA fragments are retarded in the gel at different positions according to their specific melting or partial melting temperatures (see, e.g., Myers et
25   al., Science 230, 1242, 1985). Sequence changes at specific locations can also be revealed by nuclease protection assays, such as RNase and S 1 protection or the chemical cleavage method (e.g., Cotton et al., Proc. Natl. Acad. Sci. USA 85, 4397-4401, 1985). Thus, the detection of a specific DNA sequence can be performed by methods such as hybridization, RNase protection, chemical cleavage, direct DNA
30   sequencing or the use of restriction enzymes and Southern blotting of genomic DNA.

In addition to direct methods such as gel-electrophoresis and DNA sequencing, mutations can also be detected by *in situ* analysis.

Altered levels of a GPCR also can be detected in various tissues. Assays used to detect levels of the receptor polypeptides in a body sample, such as blood or a tissue biopsy, derived from a host are well known to those of skill in the art and include radioimmunoassays, competitive binding assays, Western blot analysis, and ELISA assays.

All patents and patent applications cited in this disclosure are expressly incorporated herein by reference. The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific examples which are provided for purposes of illustration only and are not intended to limit the scope of the invention.

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EXAMPLE 1

Detection of RTA-like-GPCR activity

5   The polynucleotide of SEQ ID NO: 1 is inserted into the expression vector pCEV4 and the expression vector pCEV4-RTA-like-GPCR polypeptide obtained is transfected into human embryonic kidney 293 cells. The cells are scraped from a culture flask into 5 ml of Tris HCl, 5 mM EDTA, pH 7.5, and lysed by sonication. Cell lysates are centrifuged at 1000 rpm for 5 minutes at 4 °C. The supernatant is

10   centrifuged at 30,000 x g for 20 minutes at 4 °C. The pellet is suspended in binding buffer containing 50 mM Tris HCl, 5 mM $MgSO_4$, 1 mM EDTA, 100 mM NaCl, pH 7.5, supplemented with 0.1 % BSA, 2 μg/ml aprotinin, 0.5 mg/ml leupeptin, and 10 μg/ml phosphoramidon. Optimal membrane suspension dilutions, defined as the protein concentration required to bind less than 10 % of an added radioligand, i.e.

15   ^{125}I-labeled RTA, are added to 96-well polypropylene microtiter plates containing ligand, non-labeled peptides, and binding buffer to a final volume of 250 μl.

In equilibrium saturation binding assays, membrane preparations are incubated in the presence of increasing concentrations (0.1 nM to 4 nM) of ^{125}I ligand.

20

Binding reaction mixtures are incubated for one hour at 30 °C. The reaction is stopped by filtration through GF/B filters treated with 0.5% polyethyleneimine, using a cell harvester. Radioactivity is measured by scintillation counting, and data are analyzed by a computerized non-linear regression program. Non-specific binding is

25   defined as the amount of radioactivity remaining after incubation of membrane protein in the presence of 100 nM of unlabeled peptide. Protein concentration is measured by the Bradford method using Bio-Rad Reagent, with bovine serum albumin as a standard. The RTA-like-GPCR activity of the polypeptide comprising the amino acid sequence of SEQ ID NO: 2 is demonstrated.

30

- 55 -

EXAMPLE 2

Radioligand binding assays

5   Human embryonic kidney 293 cells transfected with a polynucleotide which expresses human RTA-like-GPCR are scraped from a culture flask into 5 ml of Tris HCl, 5 mM EDTA, pH 7.5, and lysed by sonication. Cell lysates are centrifuged at 1000 rpm for 5 minutes at 4 °C. The supernatant is centrifuged at 30,000 x g for 20 minutes at 4 °C. The pellet is suspended in binding buffer containing 50 mM Tris
10   HCl, 5 mM MgSO$_4$, 1 mM EDTA, 100 mM NaCl, pH 7.5, supplemented with 0.1 % BSA, 2 µg/ml aprotinin, 0.5 mg/ml leupeptin, and 10⁻ µg/ml phosphoramidon. Optimal membrane suspension dilutions, defined as the protein concentration required to bind less than 10% of the added radioligand, i.e. RTA, are added to 96-well polypropylene microtiter plates containing ^{125}I-labeled ligand or test compound,
15   non-labeled peptides, and binding buffer to a final volume of 250 µl.

In equilibrium saturation binding assays, membrane preparations are incubated in the presence of increasing concentrations (0.1 nM to 4 nM) of ^{125}I-labeled ligand or test compound (specific activity 2200 Ci/mmol). The binding affinities of different test
20   compounds are determined in equilibrium competition binding assays, using 0.1 nM ^{125}I- peptide in the presence of twelve different concentrations of each test compound.

Binding reaction mixtures are incubated for one hour at 30 °C. The reaction is
25   stopped by filtration through GF/B filters treated with 0.5% polyethyleneimine, using a cell harvester. Radioactivity is measured by scintillation counting, and data are analyzed by a computerized non-linear regression program.

Non-specific binding is defined as the amount of radioactivity remaining after
30   incubation of membrane protein in the presence of 100 nM of unlabeled peptide. Protein concentration is measured by the Bradford method using Bio-Rad Reagent,

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with bovine serum albumin as a standard. A test compound which increases the radioactivity of membrane protein by at least 15% relative to radioactivity of membrane protein which was not incubated with a test compound is identified as a compound which binds to a human RTA-like-GPCR polypeptide.

5

EXAMPLE 3

Effect of a test compound on human RTA-like-GPCR-mediated cyclic AMP formation

10   Receptor-mediated inhibition of cAMP formation can be assayed in host cells which express human RTA-like-GPCR. Cells are plated in 96-well-plates and incubated in Dulbecco's phosphate buffered saline (PBS) supplemented with 10 mM HEPES, 5 mM theophylline, 2 μg/ml aprotinin, 0.5 mg/ml leupeptin, and 10 μg/ml phosphoramidon for 20 minutes at 37 °C in 5% CO_2. A test compound is added and

15   incubated for an additional 10 minutes at 37 °C. The medium is aspirated, and the reaction is stopped by the addition of 100 mM HCl. The plates are stored at 4 °C for 15 minutes. cAMP content in the stopping solution is measured by radioimmunoassay.

20   Radioactivity is quantified using a gamma counter equipped with data reduction software. A test compound which decreases radioactivity of the contents of a well relative to radioactivity of the contents of a well in the absence of the test compound is identified as a potential inhibitor of cAMP formation. A test compound which increases radioactivity of the contents of a well relative to radioactivity of the

25   contents of a well in the absence of the test compound is identified as a potential enhancer of cAMP formation.

EXAMPLE 4

Effect of a test compound on the mobilization of intracellular calcium

5   Intracellular free calcium concentration can be measured by microspectrofluorometry using the fluorescent indicator dye Fura-2/AM (Bush *et al.*, *J. Neurochem.* *57*, 562-74, 1991). Stably transfected cells are seeded onto a 35 mm culture dish containing a glass coverslip insert. Cells are washed with HBS , incubated with a test compound, and loaded with 100 μl of Fura-2/AM (10 μM) for 20-40 minutes. After washing

10   with HBS to remove the Fura-2/AM solution, cells are equilibrated in HBS for 10-20 minutes. Cells are then visualized under the 40X objective of a Leitz Fluovert FS microscope.

Fluorescence emission is determined at 510 nM, with excitation wavelengths

15   alternating between 340 nM and 380 nM. Raw fluorescence data are converted to calcium concentrations using standard calcium concentration curves and software analysis techniques. A test compound which increases the fluorescence by at least 15% relative to fluorescence in the absence of a test compound is identified as a compound which mobilizes intracellular calcium.

20

EXAMPLE 5

Effect of a test compound on phosphoinositide metabolism

25   Cells which stably express human RTA-like-GPCR cDNA are plated in 96-well plates and grown to confluence. The day before the assay, the growth medium is changed to 100 μl of medium containing 1% serum and 0.5 μCi ^{3}H-myinositol. The plates are incubated overnight in a CO_2 incubator (5% CO_2 at 37 °C). Immediately before the assay, the medium is removed and replaced by 200 μl of PBS containing

30   10 mM LiCl, and the cells are equilibrated with the new medium for 20 minutes.

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During this interval, cells also are equilibrated with antagonist, added as a 10 μl aliquot of a 20-fold concentrated solution in PBS.

The ^{3}H-inositol phosphate accumulation from inositol phospholipid metabolism is
5 started by adding 10 μl of a solution containing a test compound. To the first well 10 μl are added to measure basal accumulation. Eleven different concentrations of test compound are assayed in the following 11 wells of each plate row. All assays are performed in duplicate by repeating the same additions in two consecutive plate rows.
10

The plates are incubated in a CO_2 incubator for one hour. The reaction is terminated by adding 15 μl of 50% v/v trichloroacetic acid (TCA), followed by a 40 minute incubation at 4 °C. After neutralizing TCA with 40 μl of 1 M Tris, the content of the wells is transferred to a Multiscreen HV filter plate (Millipore) containing Dowex
15 AG1-X8 (200-400 mesh, formate form). The filter plates are prepared by adding 200 μl of Dowex AG1-X8 suspension (50% v/v, water:resin) to each well. The filter plates are placed on a vacuum manifold to wash or elute the resin bed. Each well is washed 2 times with 200 μl of water, followed by 2 x 200 μl of 5 mM sodium tetraborate/60 mM ammonium formate.
20

The ^{3}H-IPs are eluted into empty 96-well plates with 200 μl of 1.2 M ammonium formate/0.1 formic acid. The content of the wells is added to 3 ml of scintillation cocktail, and radioactivity is determined by liquid scintillation counting.

25 **EXAMPLE 6**

Receptor Binding Methods

Standard Binding Assays. Binding assays are carried out in a binding buffer
30 containing 50 mM HEPES, pH 7.4, 0.5% BSA, and 5 mM $MgCl_2$. The standard assay for radioligand binding to membrane fragments comprising RTA-like-GPCR

polypeptides is carried out as follows in 96 well microtiter plates (*e.g.*, Dynatech Immulon II Removawell plates). Radioligand is diluted in binding buffer+ PMSF/Baci to the desired cpm per 50 μl, then 50 μl aliquots are added to the wells. For non-specific binding samples, 5 μl of 40 μM cold ligand also is added per well. Binding is initiated by adding 150 μl per well of membrane diluted to the desired concentration (10-30 μg membrane protein/well) in binding buffer+ PMSF/Baci. Plates are then covered with Linbro mylar plate sealers (Flow Labs) and placed on a Dynatech Microshaker II. Binding is allowed to proceed at room temperature for 1-2 hours and is stopped by centrifuging the plate for 15 minutes at 2,000 x g. The supernatants are decanted, and the membrane pellets are washed once by addition of 200 μl of ice cold binding buffer, brief shaking, and recentrifugation. The individual wells are placed in 12 x 75 mm tubes and counted in an LKB Gammamaster counter (78% efficiency). Specific binding by this method is identical to that measured when free ligand is removed by rapid (3-5 seconds) filtration and washing on polyethyleneimine-coated glass fiber filters.

Three variations of the standard binding assay are also used.

1.   Competitive radioligand binding assays with a concentration range of cold ligand vs. [125] I-labeled ligand are carried out as described above with one modification. All dilutions of ligands being assayed are made in 40X PMSF/Baci to a concentration 40X the final concentration in the assay. Samples of peptide (5 μl each) are then added per microtiter well. Membranes and radioligand are diluted in binding buffer without protease inhibitors. Radioligand is added and mixed with cold ligand, and then binding is initiated by addition of membranes.

2.   Chemical cross-linking of radioligand with receptor is done after a binding step identical to the standard assay. However, the wash step is done with binding buffer minus BSA to reduce the possibility of non-specific cross-

linking of radioligand with BSA. The cross-linking step is carried out as described below.

3.   Larger scale binding assays to obtain membrane pellets for studies on solubilization of receptor:ligand complex and for receptor purification are also carried out. These are identical to the standard assays except that (a) binding is carried out in polypropylene tubes in volumes from 1-250 ml, (b) concentration of membrane protein is always 0.5 mg/ml, and (c) for receptor purification, BSA concentration in the binding buffer is reduced to 0.25%, and the wash step is done with binding buffer without BSA, which reduces BSA contamination of the purified receptor.

EXAMPLE 7

Chemical Cross-Linking of Radioligand to Receptor

After a radioligand binding step as described above, membrane pellets are resuspended in 200 µl per microtiter plate well of ice-cold binding buffer without BSA. Then 5 µl per well of 4 mM N-5-azido-2-nitrobenzoyloxysuccinimide (ANB-NOS, Pierce) in DMSO is added and mixed. The samples are held on ice and UV-irradiated for 10 minutes with a Mineralight R-52G lamp (UVP Inc., San Gabriel, Calif.) at a distance of 5-10 cm. Then the samples are transferred to Eppendorf microfuge tubes, the membranes pelleted by centrifugation, supernatants removed, and membranes solubilized in Laemmli SDS sample buffer for polyacrylamide gel electrophoresis (PAGE). PAGE is carried out as described below. Radiolabeled proteins are visualized by autoradiography of the dried gels with Kodak XAR film and Dupont image intensifier screens.

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EXAMPLE 8

Membrane Solubilization

5   Membrane solubilization is carried out in buffer containing 25 mM Tris , pH 8, 10% glycerol (w/v) and 0.2 mM CaCl$_2$ (solubilization buffer). The highly soluble detergents including Triton X-100, deoxycholate, deoxycholate:lysolecithin, CHAPS, and zwittergent are made up in solubilization buffer at 10% concentrations and stored as frozen aliquots. Lysolecithin is made up fresh because of insolubility upon freeze-

10   thawing and digitonin is made fresh at lower concentrations due to its more limited solubility.

To solubilize membranes, washed pellets after the binding step are resuspended free of visible particles by pipetting and vortexing in solubilization buffer at 100,000 x g

15   for 30 minutes. The supernatants are removed and held on ice and the pellets are discarded.

EXAMPLE 9

20   *Assay of Solubilized Receptors*

After binding of ^{125}I ligands and solubilization of the membranes with detergent, the intact R:L complex can be assayed by four different methods. All are carried out on ice or in a cold room at 4-10 °C.).

25

1.   Column chromatography (Knuhtsen *et al.*, *Biochem. J. 254*, 641-647, 1988). Sephadex G-50 columns (8 x 250 mm) are equilibrated with solubilization buffer containing detergent at the concentration used to solubilize membranes and 1 mg/ml bovine serum albumin. Samples of solubilized membranes

30   (0.2-0.5 ml) are applied to the columns and eluted at a flow rate of about 0.7 ml/minute. Samples (0.18 ml) are collected. Radioactivity is determined

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in a gamma counter. Void volumes of the columns are determined by the elution volume of blue dextran. Radioactivity eluting in the void volume is considered bound to protein. Radioactivity eluting later, at the same volume as free ^{125}I ligands, is considered non-bound.

2. Polyethyleneglycol precipitation (Cuatrecasas, *Proc. Natl. Acad. Sci. USA 69*, 318-322, 1972). For a 100 μl sample of solubilized membranes in a 12 x 75 mm polypropylene tube, 0.5 ml of 1% (w/v) bovine gamma globulin (Sigma) in 0.1 M sodium phosphate buffer is added, followed by 0.5 ml of 25% (w/v) polyethyleneglycol (Sigma) and mixing. The mixture is held on ice for 15 minutes. Then 3 ml of 0.1 M sodium phosphate, pH 7.4, is added per sample. The samples are rapidly (1-3 seconds) filtered over Whatman GF/B glass fiber filters and washed with 4 ml of the phosphate buffer. PEG-precipitated receptor : [125] I-ligand complex is determined by gamma counting of the filters.

3. GFB/PEI filter binding (Bruns *et al.*, *Analytical Biochem. 132*, 74-81, 1983). Whatman GF/B glass fiber filters are soaked in 0.3% polyethyleneimine (PEI, Sigma) for 3 hours. Samples of solubilized membranes (25-100 μl) are replaced in 12 x 75 mm polypropylene tubes. Then 4 ml of solubilization buffer without detergent is added per sample and the samples are immediately filtered through the GFB/PEI filters (1-3 seconds) and washed with 4 ml of solubilization buffer. CPM of receptor : [125] I-ligand complex adsorbed to filters are determined by gamma counting.

4. Charcoal/Dextran (Paul and Said, *Peptides 7[Suppl. 1]*,147-149, 1986). Dextran T70 (0.5 g, Pharmacia) is dissolved in 1 liter of water, then 5 g of activated charcoal (Norit A, alkaline; Fisher Scientific) is added. The suspension is stirred for 10 minutes at room temperature and then stored at 4 °C. until use. To measure R:L complex, 4 parts by volume of charcoal/dextran suspension are added to 1 part by volume of solubilized membrane. The

samples are mixed and held on ice for 2 minutes and then centrifuged for 2 minutes at 11,000 x g in a Beckman microfuge. Free radioligand is adsorbed charcoal/dextran and is discarded with the pellet. Receptor: ^{125}I-ligand complexes remain in the supernatant and are determined by gamma counting.

5

EXAMPLE 10

Receptor Purification

10   Binding of biotinyl-receptor to GH$_4$ C1 membranes is carried out as described above. Incubations are for 1 hour at room temperature. In the standard purification protocol, the binding incubations contain 10 nM Bio-S29. ^{125}I ligand is added as a tracer at levels of 5,000-100,000 cpm per mg of membrane protein. Control incubations contain 10 μM cold ligand to saturate the receptor with non-biotinylated ligand.

15

Solubilization of receptor:ligand complex also is carried out as described above, with 0.15% deoxycholate:lysolecithin in solubilization buffer containing 0.2 mM MgCl$_2$, to obtain 100,000 x g supernatants containing solubilized R:L complex.

20   Immobilized streptavidin (streptavidin cross-linked to 6% beaded agarose, Pierce Chemical Co.; "SA-agarose") is washed in solubilization buffer and added to the solubilized membranes as 1/30 of the final volume. This mixture is incubated with constant stirring by end-over-end rotation for 4-5 hours at 4-10 °C. Then the mixture is applied to a column and the non-bound material is washed through. Binding of

25   radioligand to SA-agarose is determined by comparing cpm in the 100,000 x g supernatant with that in the column effluent after adsorption to SA-agarose. Finally, the column is washed with 12-15 column volumes of solubilization buffer+0.15% deoxycholate:lysolecithin +1/500 (vol/vol) 100 x 4pase.

30   The streptavidin column is eluted with solubilization buffer+0.1 mM EDTA+0.1 mM EGTA+0.1 mM GTP-gamma-S (Sigma)+0.15% (wt/vol) deoxycholate:lysolecithin

+1/1000 (vol/vol) 100.times.4pase. First, one column volume of elution buffer is passed through the column and flow is stopped for 20-30 minutes. Then 3-4 more column volumes of elution buffer are passed through. All the eluates are pooled.

5   Eluates from the streptavidin column are incubated overnight (12-15 hours) with immobilized wheat germ agglutinin (WGA agarose, Vector Labs) to adsorb the receptor via interaction of covalently bound carbohydrate with the WGA lectin. The ratio (vol/vol) of WGA-agarose to streptavidin column eluate is generally 1:400. A range from 1:1000 to 1:200 also can be used. After the binding step, the resin is

10   pelleted by centrifugation, the supernatant is removed and saved, and the resin is washed 3 times (about 2 minutes each) in buffer containing 50 mM HEPES, pH 8, 5 mM MgCl$_2$, and 0.15% deoxycholate:lysolecithin. To elute the WGA-bound receptor, the resin is extracted three times by repeated mixing (vortex mixer on low speed) over a 15-30 minute period on ice, with 3 resin columns each time, of 10 mM

15   N-N'-N"-triacetylchitotriose in the same HEPES buffer used to wash the resin. After each elution step, the resin is centrifuged down and the supernatant is carefully removed, free of WGA-agarose pellets. The three, pooled eluates contain the final, purified receptor. The material non-bound to WGA contain G protein subunits specifically eluted from the streptavidin column, as well as non-specific

20   contaminants. All these fractions are stored frozen at -90 °C.

EXAMPLE 11

25   *Identification of test compounds that bind to RTA-like-GPCR polypeptides*

Purified RTA-like-GPCR polypeptides comprising a glutathione-S-transferase protein and absorbed onto glutathione-derivatized wells of 96-well microtiter plates are contacted with test compounds from a small molecule library at pH 7.0 in a

30   physiological buffer solution. RTA-like-GPCR polypeptides comprise an amino acid sequence shown in SEQ ID NO:2. The test compounds comprise a fluorescent tag.

The samples are incubated for 5 minutes to one hour. Control samples are incubated in the absence of a test compound.

The buffer solution containing the test compounds is washed from the wells. Binding of a test compound to an RTA-like-GPCR polypeptide is detected by fluorescence measurements of the contents of the wells. A test compound which increases the fluorescence in a well by at least 15% relative to fluorescence of a well in which a test compound was not incubated is identified as a compound which binds to an RTA-like-GPCR polypeptide.

EXAMPLE 12

Identification of a test compound which decreases RTA-like-GPCR gene expression

A test compound is administered to a culture of human gastric cells and incubated at 37 °C for 10 to 45 minutes. A culture of the same type of cells incubated for the same time without the test compound provides a negative control.

RNA is isolated from the two cultures as described in Chirgwin *et al.*, *Biochem. 18,* 5294-99, 1979). Northern blots are prepared using 20 to 30 µg total RNA and hybridized with a ^{32}P-labeled RTA-like-GPCR-specific probe at 65 ° C in Express-hyb (CLONTECH). The probe comprises at least 11 contiguous nucleotides selected from the complement of SEQ ID NO:1. A test compound which decreases the RTA-like-GPCR-specific signal relative to the signal obtained in the absence of the test compound is identified as an inhibitor of RTA-like-GPCR gene expression.

EXAMPLE 13

Treatment of a breast tumor a reagent which specifically binds to an RTA-like-GPCR gene product

5

Synthesis of antisense RTA-like-GPCR oligonucleotides comprising at least 11 contiguous nucleotides selected from the complement of SEQ ID NO:1 is performed on a Pharmacia Gene Assembler series synthesizer using the phosphoramidite procedure (Uhlmann *et al.*, *Chem. Rev. 90*, 534-83, 1990). Following assembly and

10 deprotection, oligonucleotides are ethanol-precipitated twice, dried, and suspended in phosphate-buffered saline (PBS) at the desired concentration. Purity of these oligonucleotides is tested by capillary gel electrophoreses and ion exchange HPLC. Endotoxin levels in the oligonucleotide preparation are determined using the Limulus Amebocyte Assay (Bang, *Biol. Bull. (Woods Hole, Mass.) 105*, 361-362, 1953).

15

The antisense oligonucleotides are administered to a patient with a breast tumor. The size of the patient's breast tumor is decreased.

CLAIMS

1. An isolated polynucleotide encoding a RTA-like-GPCR polypeptide and being selected from the group consisting of:

    a) a polynucleotide encoding a RTA-like-GPCR polypeptide comprising an amino acid sequence selected from the group consisting of:

        amino acid sequences which are at least about 50% identical to the amino acid sequence shown in SEQ ID NO: 2; and
        the amino acid sequence shown in SEQ ID NO: 2.

    b) a polynucleotide comprising the sequence of SEQ ID NO: 1;

    c) a polynucleotide which hybridizes under stringent conditions to a

        polynucleotide specified in (a) and (b);

    d) a polynucleotide the sequence of which deviates from the poly-nucleotide sequences specified in (a) to (c) due to the degeneration of the genetic code; and

    e) a polynucleotide which represents a fragment, derivative or allelic variation of a polynucleotide sequence specified in (a) to (d).

2. An expression vector containing any polynucleotide of claim 1.

3. A host cell containing the expression vector of claim 2.

4. A substantially purified RTA-like-GPCR polypeptide encoded by a poly-nucleotide of claim 1.

5.   A method for producing a RTA-like-GPCR polypeptide, wherein the method comprises the following steps:

a)   culturing the host cell of claim 3 under conditions suitable for the expression of the RTA-like-GPCR polypeptide; and

b)   recovering the RTA-like-GPCR polypeptide from the host cell culture.

6.   A method for detection of a polynucleotide encoding a RTA-like-GPCR polypetide in a biological sample comprising the following steps:

a)   hybridizing any polynucleotide of claim 1 to a nucleic acid material of a biological sample, thereby forming a hybridization complex; and

b)   detecting said hybridization complex.

7.   The method of claim 6, wherein before hybridization, the nucleic acid material of the biological sample is amplified.

8.   A method for the detection of a polynucleotide of claim 1 or a RTA-like-GPCR polypeptide of claim 5 comprising the steps of contacting a biological sample with a reagent which specifically interacts with the polynucleotide or the RTA-like-GPCR polypeptide.

9.   A diagnostic kit for conducting the method of any one of claims 6 to 8.

10.   A method of screening for agents which decrease the activity of a RTA-like-GPCR, comprising the steps of:

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contacting a test compound with any RTA-like-GPCR polypeptide encoded by any polynucleotide of claim 1;

detecting binding of the test compound of the RTA-like-GPCR polypeptide, wherein a test compound which binds to the polypeptide is identified as a potential therapeutic agent for decreasing the activity of a RTA-like-GPCR.

5

11.   A method of screening for agents which regulate the activity of a RTA-like-GPCR, comprising the steps of:

10

contacting a test compound with a RTA-like-GPCR polypeptide encoded by any polynucleotide of claim 1; and

detecting a RTA-like-GPCR activity of the polypeptide, wherein a test compound which increases the RTA-like-GPCR activity is identified as a potential therapeutic agent for increasing the activity of the RTA-like-GPCR, and wherein a test compound which decreases the RTA-like-GPCR activity of the polypeptide is identified as a potential therapeutic agent for decreasing the activity of the RTA-like-GPCR.

15

20

12.   A method of screening for agents which decrease the activity of a RTA-like-GPCR, comprising the steps of:

contacting a test compound with any polynucleotide of claim 1 and

25

detecting binding of the test compound to the polynucleotide, wherein a test compound which binds to the polynucleotide is identified as a potential therapeutic agent for decreasing the activity of RTA-like-GPCR.

30   13.   A method of reducing the activity of RTA-like-GPCR, comprising the steps of:

contacting a cell with a reagent which specifically binds to any poly-nucleotide of claim 1 or any RTA-like-GPCR polypeptide of claim 4, whereby the activity of RTA-like-GPCR is reduced.

14. A reagent that modulates the activity of a RTA-like-GPCR polypeptide or a polynucleotide wherein said reagent is identified by the method of any of the claims 10 to 12.

15. A pharmaceutical composition, comprising:

the expression vector of claim 2 or the reagent of claim 14 and a pharma-ceutically acceptable carrier.

16. Use of the pharmaceutical composition of claim 15 for modulating the activity of a RTA-like-GPCR in a disease.

17. Use of claim 16 wherein the disease is bacterial, fungal, protozoan, and viral infection, pain, cancer, anorexia, bulimia, asthma, Parkinson's disease, acute heart failure, hypotension, hypertension, urinary retention, osteoporosis, angina pectoris, myocardial infarction, ulcer, asthma, allergie, multiple sclerosis, benign prostatic hypertrophy, and psychotic and neurological disorder, mental retardation, and dyskinesia.

Fig. 1

The start and stop codons are identified.

AAGGCAGCTAAGATGGGATGGGGAAGGAAACTTGAATAAATGGAGGGTTTTTAATGGGCTGA
TGCTCATGGT

GTGTCTTAAACTAAGGAGTGTAGTTAATTCTACACTTTGCCCTGAGGTCTAATTTTTTTTTT
TTTTTAAGAG

ACAGCATCTCGCTCTGTCTTCCAGGCTGGGGTGCAGTGGCGGGACCATGGCTCACTACAACC
TCAATGTGCC

AGGCTCAAGCGATCCTCCCGCCTTGGCCTCTCAAGTAGGTGGGACCACAGGTGTGTGCCACC
ATGCCTGGCT

AATTAAAAAAAAAAATTGTAGAGATGAGATCTTGCTATGTGGCCCAGGCTGGTCTCAAACTC
CTGGCCTCAA

GCAAACCCCAAAGTGCTGGGGTTACAGGCATGCACCACCACGCCCAGCCCTAAGGTCTATTG
TTTTTTTAAT

TAACTCACANNGGTTTGACAATTACAGGAGAAAAAAAAAATGATTTCAAATGTTTCTATCAA
ATAGTTTTTC

TACATCTATTGATAATAATGATTTTTCTATAATAGATGTAGAAAAAGCATTTGATAAAATTC
AATACCTCTC

CATGATTTAAAAAATAATTCAAAGCAAGATAGAAGAGAATTTTTTAACCTGATAAAGGGTAT
TTATAACAGC

TTTGTAGCTATAAAGGTGACCCTGGAGGCAGATCTGGGCTGAGTGAGTCACAGGAGTGGGGG
AAGGTTAAGC

CTCCCGGTCATGGTCTGTTACATAAAGAACCTGGAGGGGAGAGCTCTGAAGCTGGAAATTCG
AGGGGCACCC

AGGGGTCCCCCGTGGTCCCAGCAACACAGAATTTCACATTGGTACATCTTCCTCTCGTAGGG
ATGAACCAGA

CTTTGAATAGCAGTGGGACCGTGGAGTCAGCCCTAAACTATTCCAGAGGGAGCACAGTGCAC
ACGGCCTACCTGGTGCTGAGCTCCCTGGCCATGTTCACCTGCCTGTGCGGGATGGCAGGCAA
CAGCATGGTGATCTGGCTGCTGGGCTTTCGAATGCACAGGAACCCCTTCTGCATCTATATCC
TCAACCTGGCGGCAGCCGACCTCCTCTTCCTCTTCAGCATGGCTTCCACGCTCAGCCTGGAA
ACCCAGCCCCTGGTCAATACCACTGACAAGGTCCACGAGCTGATGAAGAGACTGATGTACTT
TGCCTACACAGTGGGCCTGAGCCTGCTGACGGCCATCAGCACCCAGCGCTGTCTCTCTGTCC
TCTTCCCTATCTGGTTCAAGTGTCACCGGCCCAGGCACCTGTCAGCCTGGGTGTGTGGCCTG
CTGTGGACACTCTGTCTCCTGATGAACGGGTTGACCTCTTCCTTCTGCAGCAAGTTCTTGAA

ATTCAATGAAGATCGGTGCTTCAGGGTGGACATGGTCCAGGCCGCCCTCATCATGGGGGTCT
TAACCCCAGTGATGACTCTGTCCAGCCTGACC
CTCTTTGTCTGGGTGCGGAGGAGCTCCCAGCAGTGGCGGCGGCAGCCCACACGGCTGTTCGT
GGTGGTCCTGGCCTCTGTCCTGGTGTTCCTCATCTGTTCCCTGCCTCTGAGCATCTACTGGT
TTGTGCTCTACTGGTTGAGCCTGCCGCCCGAGATGCAGGTCCTGTGCTTCAGCTTGTCACGC
CTCTCCTCGTCCGTAAGCAGCAGCGCCAACCCCGTCATCTACTTCCTGGTGGGCAGCCGGAG
GAGCCACAGGCTGCCCACCAGGTCCCTGGGGACTGTGCTCCAACAGGCGCTTCGCGAGGAGC
CCGAGCTGGAAGGTGGGGAGACGCCCACCGTGGGCACCAATGAGATGGGGGCT**TGA**GAGCCG

CCCACAGGTGCTTCCCACCTGTGCGAGCCCATGCCCTGGAGATTCCCGAGCCGTGAGC
TGCCTCCCACCTCGTCCCT

Fig. 2

MNQTLNSSGTVESALNYSRGSTVHTAYLVLS<u>SLAMFTCLCGMAGNSMVIWLLGF</u>RMHRNPF<u>C
IYILNLAAAD</u>
<u>LLFLFSMASTL</u>SLETQPLVNTTDKVHELMKRLMY<u>FAYTVGLSLLTAISTQRCLSVLF</u>PIWFK
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<u>**Transmembrane helix**</u>

SEQUENCE LISTING

<110> Bayer AG
     Bull, Christof

<120> REGULATION OF HUMAN RTA-LIKE G PROTEIN-COUPLED RECEPTOR

<130> Lio033 foreign countries

<150> 60/191,765
<151> 2000-03-24

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Val His Glu Leu Met Lys Arg Leu Met Tyr Phe Ala Tyr Thr Val Gly
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Arg Val Asp Met Val Gln Ala Ala Leu Ile Met Gly Val Leu Thr Pro
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Cys Phe Ser Leu Ser Arg Leu Ser Ser Ser Val Ser Ser Ser Ala Asn
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Pro Val Ile Tyr Phe Leu Val Gly Ser Arg Arg Ser His Arg Leu Pro
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Thr Arg Ser Leu Gly Thr Val Leu Gln Gln Ala Leu Arg Glu Glu Pro
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Glu Leu Glu Gly Gly Glu Thr Pro Thr Val Gly Thr Asn Glu Met Gly
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Ala